Acta physiol. scand. 1959. *46.* 291—297.

From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

Apparatus for Determination of the Mean Blood Flow in the Ascending Aorta of the Cat.

By

ARVID WRETLIND.

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Abstract.

A. WRETLIND. Apparatus for determination of the mean blood flow in the ascending aorta of the cat. *Acta physiol. scand.* 1959. *46.* 291—297. — A description is given of an apparatus for determination of the blood flow in the ascending aorta of the cat. The principle is a combination of measurement of the flow with differential pressure and STEPHENSON's flowmeter.

In the course of investigations on the effect of fat emulsions on the circulation, a simple flowmeter was needed which would record the blood flow in the aorta continuously, without causing any appreciable resistance or any interruption in flow. It was desirable for it to function in such a way that the variations in flow during systole and diastole would be evened out. Moreover, it should permit determination of rapid changes in the mean blood flow. A description is given in this paper of a simple flowmeter, based on a combination of the principle for determination of the flow with differential pressure, and the principle used

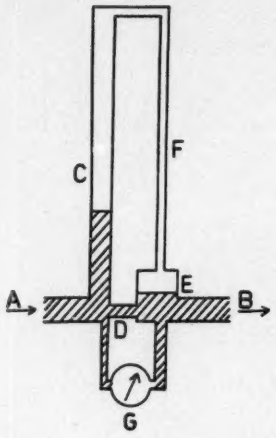
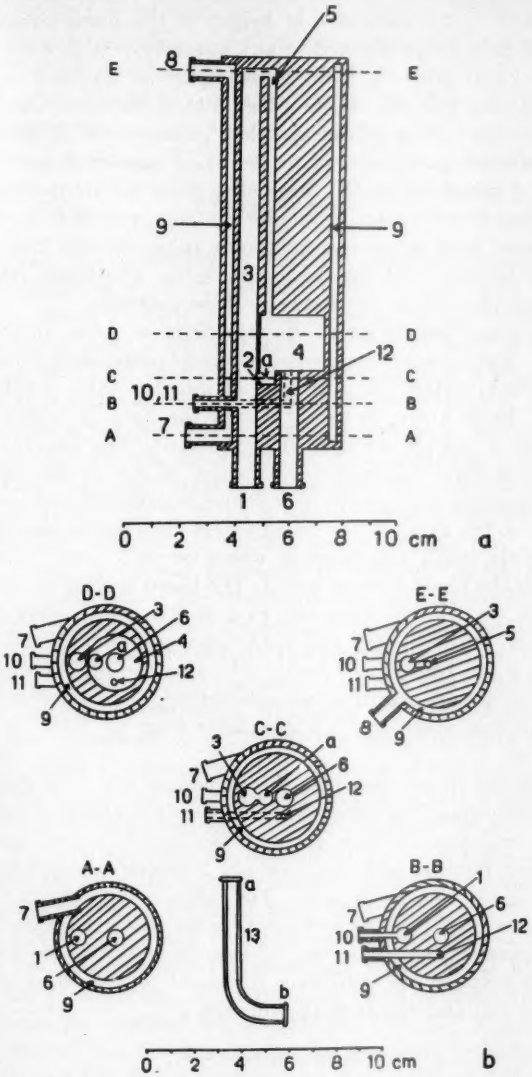


Fig. 1. *Graph showing the principle of the flowmeter.* The apparatus is coupled into the ascending aorta so that the blood from the heart runs into the apparatus at *A*, and is returned to the peripheral part of the ascending aorta via *B*.

in STEPHENSON's flowmeter (1948). The apparatus permits continuous determination of the mean flow in the ascending aorta of the cat.

Apparatus.

Principle. The principle of the apparatus is shown in Fig. 1. The blood runs through the flowmeter from *A* to *B*. A constriction is present at *D*. This implies that the pressure in the blood mass is higher before the constriction than after it. If the difference in pressure is measured before and after the constriction, it will vary in systole and diastole from a maximal value to approximately zero, which will make it far more difficult to determine the mean blood flow. Therefore, on the pressure side, there is a vertical tube, *C*, in which the blood is collected that has not run through the constriction. The greater the flow, the higher does the blood rise in *C*, and in this way the greater is the amount which runs through the constriction at *D*. The blood rises in *C* until equilibrium has taken place between the height of the blood column in *C* and the flow via *D*. The constriction (*D*) is adjusted so that the blood in *C* rises to a suitable height for recording. The difference in blood pressure in *C* and *E* is measured by the differential-pressure meter, *G*.



Figs. 2 a and b. *Drawing of the flowmeter.* Fig. 2 a shows the vertical projection, and Fig. 2 b the different sections in 2 a. — The blood flows in at 1 and rises in 3, and also enters, via 2, into 4, whence it returns to the peripheral part of the blood vessel via 6. In Fig. 2 b, the detail 13 shows the appearance of one of the glass cannulas introduced into the aorta. The end, *a* (13) is connected by a rubber tube to 1 and 6, respectively, in Fig. 2 a. For further details: see text.

To prevent the difference in height of the blood columns in *C* and *E* from being affected to any appreciable degree by variations in blood pressure, *C* is connected by an air-filled tube, *F*, with the reservoir, *E*, on the other side of the constriction (*D*). This has the further advantage that pulsations in *C* are transmitted almost quantitatively to the blood mass in *E*, and thus to the blood peripheral to the flowmeter. Since we are dealing with a pulsating blood stream, it implies that the level in *C* fluctuates. The lowest level gives the minimum value for the flow in *D*, and the highest level the maximum value. The exact value for the mean blood flow lies between these extremes.

Apparatus. The design of the flowmeter is shown in Fig. 2 a and 2 b. The apparatus is made entirely of transparent Plexiglass.

The blood enters via 1 (Fig. 2 a) into the tube, 3 (diameter 7 mm). From there, it runs through the hole, 2, which has a diameter of exactly 3.0 mm. It is the size of the hole (2) which at a given flow determines the height of rise in the tube (3). The dimensions given have been found suitable for cats weighing from 3—4 kg. From 2, the blood enters the circular reservoir, 4, through the small depression, *a*, which serves to damp the movements of the blood flowing into 4. The blood is then led from the reservoir (4), via the tube (6), back to the aorta. Through the connexion (5) between 3 and 4, the movements of the blood column in 3 are transmitted to the blood in the reservoir (4). The flowmeter is enclosed in a water jacket (9), through which water with a temperature of 37° C circulates from the inlet (7) to the outlet (8).

When the blood runs into the apparatus via 1, it rises in 3, until equilibrium has taken place between the height of the blood column in 3 and the flow via 2. If the flow increases, the blood in 3 rises further, and the flow via 2 increases simultaneously, owing to the rise in pressure. The relation between the height of the rise in *C* (Fig. 1) and the flow at *D* is exponential. With this apparatus, there is — purely experimentally — the following relation between the difference in height of the blood levels in 3 and 4 (H) and the flow (F) through 2:

$$H = \text{constant} \cdot F^{a}.$$

The value of *a* has been found experimentally to be 2.8. The constant is $10^{-5.0}$ if H is given in mm and F in ml per min.

Determination of the pressure difference, or difference in levels,

takes place by coupling a differential-pressure meter (Schaevitz Type P-476-A 10 Pressure Transducer[1]) to outlets 10 and 11, which are connected to the tube (3) and, via 12 to the reservoir (4), respectively.

Experimental.

The following procedure is used for coupling the apparatus to the ascending aorta of the cat. Two cannulas with the appearance shown by 13 (Fig. 2 b) are coupled to the tubes, 1 and 6, by rubber tubes 3 cm in length. The curved part, *b*, of both cannulas faces each other, which implies that they overlap, since the distance between 1 and 6 is less than the length of *b*. A series of cannulas of varying calibre should be available, so that those with the best fit can be chosen for each experiment. The apparatus and cannulas are filled with physiologic saline, so that the level of the fluid in 3 and 4 reaches about 2 mm above the hole (2). The rubber tubes below 1 and 6 are clamped.

The thorax of the cat is opened transversely in the fifth to sixth intercostal space. The vessels are then more easily accessible than if the thorax is opened by splitting the sternum longitudinally. After incising the pericardium, the ascending aorta and superior and inferior venae cavae are exposed. 2 ml of a 5 per cent heparin solution is given intravenously. The caval veins are clamped, and after a few seconds the flow through the ascending aorta is minimal. The aorta is then clamped at the level of the origin of the innominate artery, and the ascending aorta is half-severed at a site halfway between the heart and the origin of the innominate artery. The two cannulas are introduced into the aorta, so that the blood will flow through the apparatus as already described. The clamps are then removed from the vessels and from the rubber tubes below 1 and 6. Clamping of the vessels and insertion of the cannulas into the ascending aorta should not take more than 1—2 minutes. As a rule, the blood pressure and flow are suitable for starting the experiment after 5—10 min. Administration of blood or dextran may be necessary to compensate for the loss of blood.

Calibration of the apparatus is carried out by allowing the blood to flow through it at different rates. A curve for the rela-

[1] Schaevitz Engineering, P. O. Box 505, *Camden 1*, N. J., U. S. A.

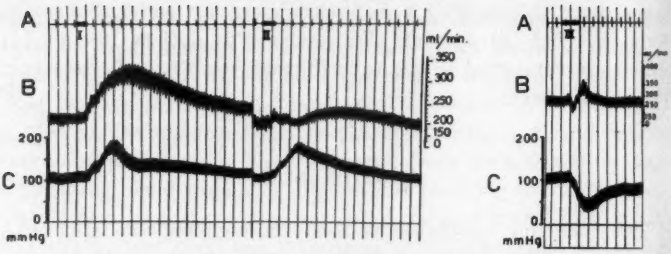


Fig. 3. *Recording of the blood flow in the ascending aorta of the cat.* Weight of cat 2.7 kg. Dial anesthesia, artificial respiration and open thorax. *A* is the time marking (10 sec) and injection signal. *B* denotes the flow in the ascending aorta. *C* is blood pressure. At the injection signal I, 10 μg of adrenaline, II, 40 μg of noradrenaline and at III, 8 μg of acetylcholine were given intravenously.

tion between the flow (F) and the difference in height of the blood columns (H) in 3 and 4 is plotted from the values then obtained.

Examples of the use of the flowmeter are shown in Fig. 3.

Discussion.

A number of types of flowmeter based on the differential-pressure principle exist (DE BURGH DALY 1926, BROEMSER 1928, WAGONER and LIVINGSTON 1928, FRANK 1929, 1930, JOHNSON and WIGGERS 1937, LAWSON and HOLT 1939, ECKSTEIN *et al.* 1947). Their drawback is that, when used in the aorta, the mean blood flow cannot be determined without planimetric calculation of the flow curves (HODGSON 1929). In the apparatus described here, this drawback has been eliminated by arranging, on the pressure side, a space for expansion, in which the blood rises until equilibrium occurs between the height of the rise and the flow. The increase in resistance in the blood stream amounts to the height of the rise of the blood in the tube, 3 (Fig. 2 a), *i. e.*, to about 5 cm water or 4 mm Hg, and can therefore be regarded as of no practical consequence.

Fig. 3 shows that an adequate recording of the flow in the aorta is obtained with this apparatus. The marked difference in the effect of various drugs on the blood flow and blood pressure can be inferred from these figures. These few examples demon-

strate the suitability of the flowmeter in pharmacological experiments, when it is to be determined whether the changes in blood pressure are due to an effect on the heart, or to an influence on the peripheral vessels.

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A Method for the Assay of 'Slow Reacting Substance'.

By

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Received 5 January 1959.

Abstract.

CHAKRAVARTY, N. A method for the assay of 'slow reacting substance'. *Acta physiol. scand.* 1959. *46*. 298—313. — A method is described for the biological assay of 'slow reacting substance' ('SRS') which appears in anaphylactic reaction. The log-dose response of 'SRS' on the guinea pig ileum has a linear regression. Using a four-point design of experiment, it is therefore possible to assay 'SRS' against a standard. It is shown by an analysis of variance that the method gives a reliable estimate of 'SRS' within relatively small limits of error.

A 'slow reacting substance' ('SRS') is known to occur in anaphylactic reaction in addition to histamine (KELLAWAY and TRETHEWIE 1940, BROCKLEHURST 1953). An investigation has been in progress in this laboratory for some time on the quantitative relationship of the two substances in the process. In the course of this investigation it was found necessary to develop a method for the assay of 'SRS'. It will be shown here that it is possible to assay 'SRS' on guinea pig ileum in arbitrary units within relatively narrow limits of error.

The term 'SRS' has been used in this communication specifically for the 'slow reacting substance' that occurs in anaphylactic

reaction, unless otherwise stated. The chemical nature of 'SRS' is not yet known, but our observations (to be published) indicate that this additional smooth muscle stimulating principle is a lipid soluble acid. It is apparently different from other known substances producing a slow contraction of guinea pig ileum.

Material and Methods.

'SRS' was produced by incubating pieces of sensitized guinea pig lung in Tyrode solution with antigen at 37° C. Further details of this method will soon be presented in a separate communication. Both histamine and 'SRS' diffused out, following anaphylactic reaction, into the Tyrode solution in which the tissues were suspended and could be tested directly in the solution. However, for most of the procedures reported here, the samples were freeze-dried and extracted with 80 per cent alcohol.

'SRS' was assayed on guinea pig ileum in the presence of 1.5×10^{-6} M atropine and enough antihistaminic to block the effect of histamine in the test solution. The amount of histamine (base) in the samples added to the bath was usually less than 0.3 μg and 10^{-7} to 10^{-6} M mepyramine in the Tyrode solution was sufficient to block its effect on the gut. The test bath was set up in the same way as for the histamine assay; in fact both histamine and 'SRS' can be assayed in the same bath. A 4 ml bath with a frontal writing lever was used, the sweep of the lever being restricted to within 30° from the horizontal. The contractions were magnified 10 to 15 times on the record. The Tyrode solution bathing the intestine was oxygenated by bubbling either air or oxygen through the bath. The temperature of the water bath was maintained at either 37° or 30° C. Disturbing spontaneous contractions were less likely to occur at the lower temperature, and both the 4-point assays of 'SRS' reported here were carried out at this temperature.

The standard.

The lungs from guinea pigs sensitized with egg albumin were removed and cut into small pieces weighing about one to two mg. The lung pieces were washed and incubated in Tyrode solution (10 ml/g tissue) with one mg per ml antigen for 10 minutes at 37° C. The incubation fluid containing 'SRS' from the lungs

Fig. 1. Response of guinea pig ileum to 'SRS'. 12 units of 'SRS' were added to the bath for each contraction. The latency between application of 'SRS' and contraction is shown under the figure. 1.5×10^{-6} M atropine and 7.5×10^{-6} M mepyramine were added to the bath. Time scale is shown in minutes. Bath temp. 37° C.

of 15 to 20 guinea pigs was freeze-dried and extracted with 80 per cent ethyl alcohol. The alcohol extract was dried in nitrogen atmosphere under reduced pressure at 50 to 55° C temperature. The material extracted with alcohol was divided into equal parts and stored as dry powder at — 20° C in vacuum in the freeze-box. Under these conditions the activity of 'SRS' was well preserved. Before use the material in a tube was dissolved in distilled water; during the test procedure it was kept cold (in ice-water) and protected from light.

When the first standard preparation was tested on guinea pig ileum in the presence of atropine and mepyramine, 0.1 ml of the Tyrode solution, corresponding to 10 mg lung tissue (wet weight) and its alcohol extract, produced a definite 'SRS' type (*vide infra*) of contraction and was defined as a unit of 'SRS'. Subsequently, 3 more standards were prepared. Their 'SRS' activity was determined against the first standard on the guinea pig ileum and all 'SRS' values have been expressed in units based on the first standard as defined above. The threshold response of different guinea pig ileums to the standard preparations varied from 0.5 to 4 units.

Response of guinea pig ileum to 'SRS'

In the guinea pig ileum 'SRS' produces a characteristic contraction that is different from histamine-induced or other similar contractions. The contraction starts after a longer latency and is of a slower type as illustrated in Fig. 1. It usually starts

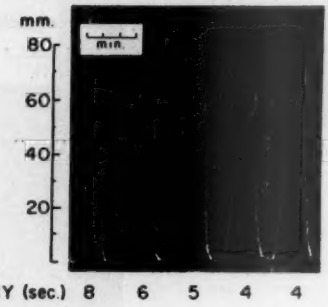

Fig. 2. Initial sensitization of guinea pig ileum to 'SRS'. The same dose (10 units) was added to the bath for each contraction. Note the increasing height of the record and the decreasing latency. 1.5×10^{-4} M atropine and 10^{-7} M mepyramine in bath. Temp. 37° C.

after a latency of 5 to 30 sec, slowly rising to a peak in 1 to 3 min, and the gut relaxes slowly with repeated washing. The latency varies inversely with the concentration of 'SRS' in the solution and also varies in different intestines.

Intestines from different guinea pigs may vary widely in their sensitivity, and the sensitivity of the same intestine is usually low in the beginning and increases considerably after exposure to a few doses of 'SRS'. If these facts are not taken into account, tests for 'SRS' may give erratic results. It was, however, possible to overcome these difficulties by using a standard for reference and by producing repeated 'SRS' contractions of the gut until it was sensitive to small doses of the standard. The usual procedure was as follows: At first, after the test-bath was set up, a few doses of histamine were added to the bath. When the intestine responded more or less regularly to small doses of histamine, the solution (Tyrode) bathing the intestine was changed over to one containing an antihistaminic; samples containing 'SRS' now produced the characteristic response. After a few such contractions the intestine was ready for the testing of unknown samples of 'SRS'. The interval between the application of consecutive doses of 'SRS' was 3 to 5 min. Fig. 2 shows the sensitization of guinea pig ileum to 'SRS'. After the inital stage of rapid increase in sensitization a stage may be reached when uniform responses are obtained. Fig. 1 shows the last phase of the initial sensitization

Fig. 3. Sensitization of guinea pig ileum to histamine after 'SRS' contraction, At the arrow ether-extracted 'SRS' was added to the bath. H = contraction caused by 0.9 μg histamine (base). Interval between histamine responses: 3—3½ minutes. 1.5×10^{-3} M atropine and 7.5×10^{-6} M mepyramine in bath. Temp. 37° C.

followed by uniform responses to the same dose of 'SRS'. However, as in histamine assay, gradual changes in sensitivity over longer period of time are more common (*vide infra*).

One of the properties of 'SRS' is to sensitize the guinea pig ileum to histamine, as may be seen in Fig. 3; 0.9 μg histamine produced a small contraction in the presence of mepyramine in the bath. The response to the same dose of histamine was markedly potentiated by a previous contraction produced by ether-extracted 'SRS'. This property must be kept in mind in testing samples containing histamine for 'SRS'. In the course of the assay it may be necessary to add more antihistaminic to the Tyrode solution to block the effect of any histamine that may be present in the test samples. It is usually possible to add relatively large amounts of antihistaminic after a few 'SRS' responses without producing nonspecific depression of the guinea pig gut.

Assay of 'SRS'.

The assay of 'SRS' with repeated graded responses is based on a linear log-dose response relationship. That such a relation exists in the case of 'SRS'-induced contraction of guinea pig

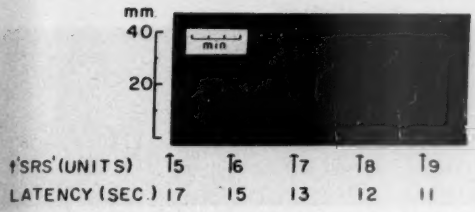

Fig. 4. Graded responses of guinea pig ileum to increasing doses of 'SRS'. Note also the decreasing latency with increasing dosage and the height of contraction. Five to nine units of 'SRS' were added to the bath at the arrows. The contractions between the 'SRS' responses were produced by histamine. 1.5×10^{-4} M atropine and 1.3×10^{-7} M mepyramine in bath. Temp. 37° C.

ileum is shown in Fig. 4 and 5. The contraction produced by the consecutive application of 5 increasing doses of 'SRS', ranging from 5 to 9 units, is shown in Fig. 4. When the recorded heights of these contractions are plotted against log-dose the linear relationship becomes evident (see Fig. 5).

A four-point design (GADDUM 1953 a, p. 496) was chosen for the assay of 'SRS' using dosage within the linear log-dose response range. The actual assay was carried out with four known doses as described below but two of them were treated as 'unknown' to determine the accuracy of the estimate.

As it is essential in an assay of this type to compute the various sources of variation in the same experiment, the procedures and calculations for such an experiment are given below in some details. This is referred to as Expt. 1.

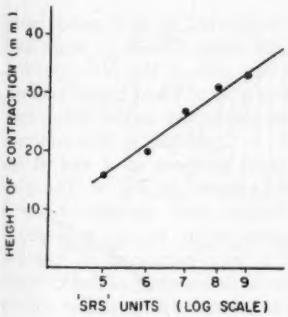

Fig. 5. Height of contraction of guinea pig ileum (from the illustration in fig. 4) plotted against the logarithm of dose of 'SRS'. The abscissa shows the actual doses on the log scale. Note the linear relationship.

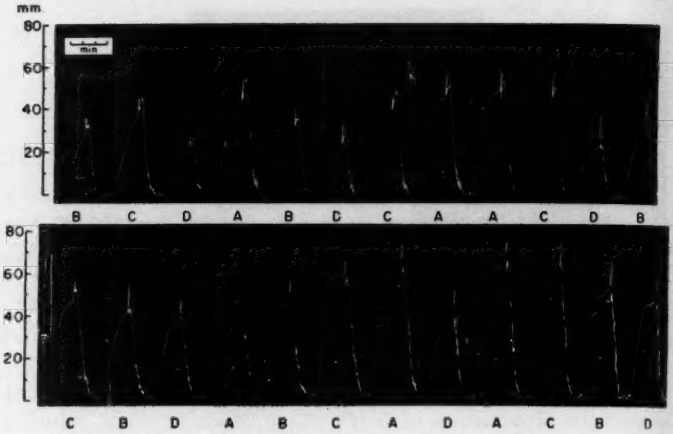

Fig. 6. Assay of 'SRS' on guinea pig ileum. Four doses of 'SRS' A, B, C, D, were added to the bath in six randomised groups giving 24 contractions. A = 8.4 units, B = 4.2 units, C = 6.72 units and D = 3.36 units. For the calculations in the text A & B were regarded as standard and C & D as 'unknown'. The ratio of the higher dose to the lower dose is the same in both cases *i. e.* A/B = C/D = 2. A dose of 'SRS' was added to the bath every 5 minutes; it was washed out after contact with the gut for 2 minutes. The drum was stopped in between the 'SRS' responses. Weight of guinea pig: 340 g (♂). Bath: Temp. 30°, 1.5×10^{-6} M atropine and 3×10^{-7} M mepyramine. Towards the end of the test the contractions rose more rapidly to a peak, especially with the higher doses. Estimated value of unknown in this experiment was 74.5 per cent of the standard with fiducial limits (P = 0.01) of 67.6 and 82 per cent. True value of the 'unknown' was 80 per cent of the standard.

A male guinea pig weighing 340 g was killed by a blow on the head, and a segment (2—2.5 cm) of ileum about 20—30 cm from the ileocaecal junction was mounted in the bath. After initial histamine contractions and preliminary sensitization to 'SRS' the assay was commenced as described hereafter.

Four doses of standard 'SRS' solution designated A, B, C and D were chosen as follows: A = 8.4 units, B = 4.2 units, C = 6.72 units and D = 3.36 units. C and D containing 80 per cent of the activity of A and B, respectively, were treated as 'unknown' and A and B as standard. The doses were so chosen that the ratio of the higher to the lower dose was the same in both cases: A/B = C/D = 2. Actually, the volumes of C and D in this experiment was the same as those of A and B respectively. The performance of the assay is shown in Fig. 6. The four doses comprising a group were added to the bath successively in 6 groups, the order of application in a group being chosen at random (from tables of random numbers). Each dose remained in contact with the gut for 2 min, and the successive doses were added every 5 min, thus allowing sufficient time for relaxation of the gut. The results

Table I.

Responses of guinea pig ileum to four doses of 'SRS' applied in six randomized groups (Expt. I).

Dose (units)	Log dose	Heights of Contraction (mm) Groups						Total
		1	2	3	4	5	6	
8.4 (A)	0.924	41	46	49	59	64	67	326
4.2 (B)	0.623	27	32	33	41	45	50	228
6.72 (C)	0.827	36	37	45	47	56	61	282
3.36 (D)	0.526	20	22	27	37	39	47	192
	Total	124	137	154	184	204	225	1,028

of the assay are presented in Table I. There is an apparent difference in the size of the effect produced by the different doses. The effect also increased in successive groups with all the doses. This is more clearly seen in Fig. 7. The mean latency of the responses to the higher and the lower doses of the standard was 18.8 and 23.0 sec, the corresponding values for the 'unknown' being 20.8 and 24.0 sec respectively. There was not only a difference in the latency with the different doses (a high dose having lower latency) but there was also a definite decrease in the latency as the sensitivity increased with time.

Fig. 8 shows the graphical determination of the potency of the 'unknown', *i. e.* C or D · M, the log ratio of potency of standard and 'unknown' works out graphically at 0.13. So, standard/'unknown' = antilog of 0.13 = 1.35.

However, in order to determine the error of the estimate of M its value has been calculated from the simplified formula (GADDUM

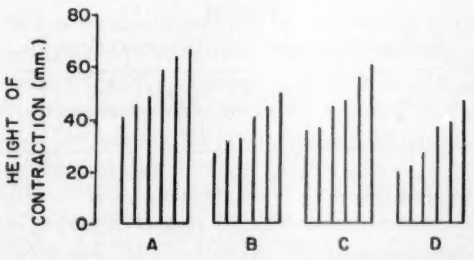

Fig. 7. Heights of contraction of the guinea pig ileum in response to the 4 doses of 'SRS': A, B, C, D in the 6 successive groups (from the experiment shown in fig. 6). Note the gradual and more or less uniform increase in sensitivity with all 4 doses.

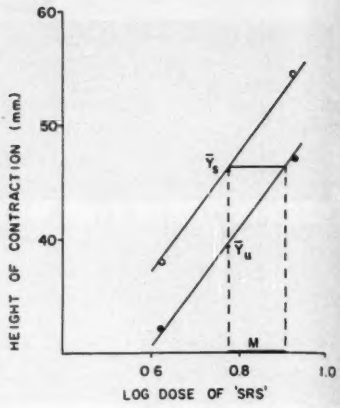

Fig. 8. Graphical determination of M, the log ratio of potency of standard to unknown. The upper line is drawn between the mean responses to the higher and lower doses of the standard and lower line between similar responses to the 'unknown'. $\bar{y}_s$ = mean of all standard responses and $\bar{y}_u$ = mean of all unknown responses. The antilog of the horizontal distance between the two lines is a measure of the ratio of potency of standard to 'unknown'. Here M = 0.13 and its antilog = 1.35, and graphically, the ratio of potency of standard to 'unknown' = 1.35.

1953 b): $M = (\bar{y}_s - \bar{y}_u)/b$ as the doses measured in volumes are the same here for the standard and the 'unknown'; $\bar{y}_s$ and $\bar{y}_u$ in the equation are the mean responses of the standard and the 'unknown' and b is the slope of the regression line. Substituting these values, M works out at 0.12815, the antilog of which is 1.343.

Analysis of variance.

In order to determine the different sources of variation in the response and to assess their significance, a standard analysis of

Table II.

Analysis of variance of 'SRS' assay (Expt. I).

Source of variation	Sum of squares	Degrees of freedom	Variance	F	P
A. Between groups	1,956.84	5	391.37	121.17	< 0.001
B. Between standard and 'unknown'.....	266.67	1	266.67	82.56	< 0.001
C. Regression	1,472.67	1	1,472.67	455.93	< 0.001
D. Deviation from parallelism .	2.67	1	2.67	< 1	—
E. Error (T — (A + B + C + D))	48.49	15	3.23	—	—
Total (T)	3,747.34	23			

variance of the data in Table I was computed (SNEDECOR 1956, p. 237). It may be seen from Table II that there is a highly significant difference between the standard and the 'unknown' and a highly significant regression; there is an extremely good parallelism ($F < 1$). The difference between the groups is also highly significant.

Limits of error of M.

The limits of error (s_M) of the estimate of M could be calculated after taking into consideration the variance of the regression (b). As Finney's g-criterion gave a value of $g = 0.01$ it has been possible to calculate the approximate value of s_M from a simplified formula (BURN, FINNEY and GOODWIN 1950, p. 83) which can be used more conveniently in the following form (GADDUM 1953 a, p. 497) for the present experiment:

$$s_M^2 = (1 + M^2/d^2)\ V/b^2$$

V being the mean of the variances of the four estimates of the mean effect and d the log ratio of potency of the higher to the lower dose. Substituting the respective values, we obtain $s_M = 0.01418$, and at 99 per cent probability the limits of error for M are $M \pm 2.947\ s_M$. So, the calculated value of the 'unknown' expressed as percentage of the standard is 74.5 per cent with fiducial limits ($P = 0.01$) of 67.6 and 82 per cent, thus including the true value of 80 per cent.

Test of linearity of regression.

In order to test for the linearity of the regression, it is necessary to have more than two points on the line. Since the assay presented in Table I was actually conducted with four known doses the linearity of regression could be tested by using all four doses and their responses to obtain the regression equation. It is then necessary to take into consideration the changes in the sensitivity of the intestine which responded to the same dose of 'SRS' by a progressively increased height of contraction during its successive applications (see Fig. 7). Using a covariance analysis (SNEDECOR 1956, p. 394) it was found that the regressions for the rise in sensitivity were parallel for the four doses ($F = 2.58$, d. f. = 3; 16). After adequate adjustment of the values for the change

Table III.

'SRS' assay on guinea pig ileum.

Expt. No.	True value of 'unknown' as % of 'standard'	Ratio of higher to lower dose	Difference in activity between 'unknown' and 'standard'	Difference between the groups	Regression	Deviation from parallelism	Estimated activity %	P 0.01 limits of estimated activity %	$\lambda = a/b$
1	80	2:1	H. S.	H. S.	H. S.	N. S.	74.5	67.6—82.0	0.028
2	80	8:5	H. S.	H. S.	H. S.	N. S.	74.0	67.7—81.2	0.027

H. S. = highly significant, $P < 0.001$.
N. S. = not significant, $P > 0.05$.

of sensitivity and introducing the deviation from the common regression it was found that there was no significant deviation from linearity (Ostle 1954, p. 154) for the log-dose response regression of 'SRS' during the assay ($F = 2.60$, d. f. = 2; 19).

In another 'SRS' assay conducted in the same way (Expt. 2) an analysis of variance of the data yielded very similar results. The four doses of 'SRS' used for the second experiment were 5.6, 3.5, 4.48, and 2.8 units, the last two being regarded as the 'unknown' for the assay. The ratio of the higher to the lower dose was 8:5 in this case. The results of the assay along with those of Expt. 1 are summarised in Table III.

Changes in the sensitivity of the preparation.

It is evident from Fig. 7 that the same dose produced a higher response in successive applications. The nature of this rise in sensitivity is more clearly shown by plotting the mean response in each group. Fig. 9 shows that in both experiments there was a rather uniform rise in sensitivity. This also accounts for the highly significant difference between the groups in the analysis of variance (Table II). Owing to this change in sensitivity it was necessary to use the standard and 'unknown' solutions in several groups in random sequence.

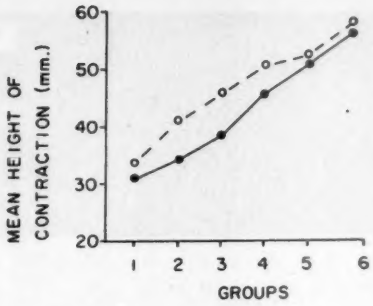

Fig. 9. Changes in the sensitivity of guinea pig ileum to 'SRS' during the assay as indicated by the mean response in the successive groups. Expt. 1: continuous line. Expt. 2: dotted line. Note the gradual increase in sensitivity in both the cases.

Assay of 'SRS' in the presence of compound 48/80.

In our experiments on the appearance of 'SRS' from perfused cat's paw induced by compound 48/80 (CHAKRAVARTY, HÖGBERG and UVNÄS 1959), it was necessary to determine if small amounts of compound 48/80 could influence the response of the ileum. PATON (1951) reported that compound 48/80 could antagonize the effect of 'slow contracting substance' on the guinea pig ileum. We tested the effect of compound 48/80 on both histamine and 'SRS' responses. The effect on the histamine response was not pronounced. In a 4 ml bath 1 to 2 μg compound 48/80 had no appreciable effect, while 5 to 10 μg caused slight tendency to sensitization; 25 to 50 μg produced some sensitization and occasionally irregular contractions. The effect of compound 48/80 on the 'SRS' response is illustrated in Fig. 10. 'SRS' from sensitized guinea pigs was used following extraction in 80 per cent alcohol — the same preparation referred to as 'standard' here. The 'SRS' response was not influenced by 1 to 5 μg compound 48/80; 10 μg caused slight reduction of the response while 25—100 μg reduced the response more appreciably. Recovery followed washing in 15 to 20 min after moderate doses. Alcohol extracted 'SRS' from cat's paw was tested in a similar manner for any antagonising effect of compound 48/80 with the same results. It may be seen from Fig. 10 that the effect of compound 48/80 persisted and the succeeding 'SRS' response of the gut, where no compound 48/80 was added, was also reduced. In an

Fig. 10. Effect of compound 48/80 on 'SRS' response. From 1 to 25 μg compound 48/80 was added to the bath one-half minute before the addition of 'SRS'. Successive doses were applied every 5 minutes. The drum was stopped between responses. Bath: temp. 30° C, atropine 1.5×10^{-3} M, mepyramine 6.25×10^{-7} M. Note depression of 'SRS' response with higher doses of compound 48/80.

assay, therefore, where standard and unknown samples are added alternately, both the responses would be reduced by these doses of compound 48/80 and the error introduced would not be high. Actually, the concentration of compound 48/80 that could be present in our experiments in the perfusate tested for 'SRS' was much lower and, judging from these results, would not influence the response of the gut to 'SRS'.

Discussion.

Although it has been known for several years that 'slow reacting substance' occurs in anaphylactic reaction, no quantitative data on 'SRS' have been available so far. With the use of a standard and an assay technique on the four-point design on guinea pig ileum it is now possible to give quantitative values for 'SRS' along with histamine released under various experimental conditions. Such investigations have been carried out showing a striking correlation between the appearance of histamine and 'SRS' caused by anaphylaxis or by compound 48/80. The results of the investigations will be reported in separate communications.

It has been shown, using a four-point design of assay, that

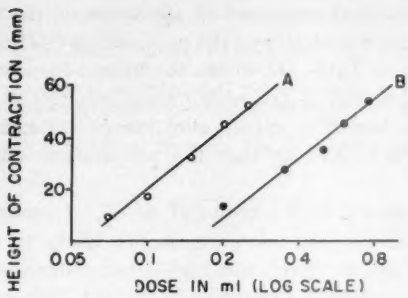

Fig. 11. Log-dose response regression for (A) 'SRS' from perfused cat's paw following 48/80 injection and for (B) 'SRS' from sensitized guinea pig lung in anaphylactic reaction. Both were extracted with 80 per cent alcohol before testing on guinea pig ileum. Five doses (ml) of each were added to the bath in the following order: 0.15 A, 0.5 B, 0.1 A, 0.35 B, 0.2 A, 0.6 B, 0.25 A, 0.75 B, 0.07 A, 0.2 B, and the heights of the contractions were plotted against the log dose. Note that the two regression lines are parallel.

the concentration of 'SRS' in unknown samples can be estimated on the guinea pig ileum within rather small limits of error. In both the experiments the actual concentration of the 'unknown' was 80 per cent of the standard. The estimated values were 74.0 and 74.5 per cent with fiducial limits ($P = 0.01$) of 67.7—81.2 per cent and 67.6—82 per cent respectively. The error is comparable to that of histamine in SCHILD's (1942) assay or of posterior pituitary extract assayed by HOLTON (1948) on the same principle on rat's uterus. More recently ROCHA E SILVA (1952) has shown that another smooth muscle contracting principle, bradykinin, can be satisfactorily assayed on the guinea pig gut in a similar manner.

It has been shown in the present experiments that the log-dose response regression for 'SRS' on guinea pig ileum shows no significant deviation from linearity thus making the assay possible on the four-point design. Table III shows that in both the assays the regression and the difference between the standard and the 'unknown' were highly significant and that there was no significant deviation from parallelism. The difference between the groups was also highly significant due to changes in the sensitivity of the preparation. But an experimental design of the type followed here with several random sequence of four doses eliminated this source of variation to a large extent. The ratio of standard

deviation of individual responses to the slope of the regression is a measure of the variability of the preparation (GADDUM 1931). The last column in Table III shows the values for $\lambda = s/b$, *i. e.* ratio of the square root of the error ('mean square') to the slope of the regression line. The values were low (0.027 and 0.028) in both experiments indicating that the preparation was suitable for 'SRS' assay.

It has been shown that compound 48/80, if present in low concentrations in the test solutions, is not likely to affect the response of the gut to 'SRS', but higher concentrations of compound 48/80 can reduce the response of the gut. This is true with regard to 'SRS' both from sensitized guinea pig's lung and from cat's paw. In fact 'SRS' from the two sources appears to be very similar in properties (*vide infra*).

We have reported elsewhere on the appearance of 'SRS' from perfused cat's paw following injection of compound 48/80 (CHAKRAVARTY, HÖGBERG and UVNÄS 1959). When this 'SRS' from cat's paw and 'SRS' appearing in anaphylactic reaction from sensitized guinea pig's lung were added alternately to the test bath, the two log-dose response regressions were parallel (Fig. 11). It was therefore possible to assay 'SRS' from cat's paw using the same standard prepared from sensitized guinea pig lungs. Moreover, 'SRS' from the two sources behaves rather similarly in paper chromatography. It seems from these observations that they are either the same or similar substances.

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The Effect of Acetylcholine and Prostigmine on Catechol Amine Excretion in Rats.

By

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Received 7 January 1959.

Abstract.

GRANITSAS. A. N. The effect of acetylcholine and prostigmine on catechol amine excretion in rats. *Acta physiol. scand.* 1959. *46*. 314—318. — Acetylcholine in doses of 0.05—1 mg s. c. per rat increased the noradrenaline (NA) output in urine without causing a significant change in the adrenaline (A) output. Prostigmine also increased the NA excretion.

Acetylcholine is known to be a powerful stimulant of adrenal medullary secretion. FELDBERG, MINZ and TSUDZIMURA (1934) and BUTTERWORTH and MANN (1957) have shown that it depletes the adrenal medulla when injected i. v. in repeated doses in atropinized cats. However, there is no information concerning its effect on catechol amine excretion. Noradrenaline (NA) and adrenaline (A) are normal constituents of the urine, NA representing 60—90 % of total catechol amines in urine (EULER, HELLNER-BJÖRKMAN and ORWÉN 1955). Normally the origin of urine NA is chiefly extra-medullary, since after adrenalectomy the excretion of NA is not decreased (EULER, FRANKSSON and HELLSTRÖM 1954, CRAWFORD and LAW 1958). By analysing the urine for NA and A it was hoped to obtain information as to whether acetylcholine acts solely on the suprarenal gland or whether the adrenergic nervous system is involved as well.

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Material and methods.

Male adult rats, weighing 250—260 g were used. The animals were kept in metabolic cages in groups of 5 animals per cage. All the animals were fed ad libitum a standard stock food. The urine of 15 rats was pooled for determination of catechol amines. In each experiment urine was collected during a control period between 9—11 a. m. After the drug injection urine was collected between 11 a. m. and 1 p. m. and 1—3 p. m. Care was taken to secure uniform collection by slight pressing of the abdomen at the end of each period in order to produce reflex urination. The urine was collected in plastic bottles containing 0.5 ml 3 N H_2SO_4. Acetylcholine in 0.9 % NaCl was injected subcutaneously in varying doses on 4—5 occasions in a volume of 0.2 ml per animal. Control injections of saline were also made. The doses of acetylcholine and prostigmine used are expressed per animal of 250 g body weight.

The determination of catechol amines was performed according to EULER and LISHAJKO (1959). The results are expressed in ng per kg body weight of rat per hour (1 ng = 0.001 μg).

Results.

After acetylcholine administration, salivation, lacrymation and piloerection was noticed. This effect lasted from 5—40 min depending on the dose (0.05—1 mg). The excretion of catechol amines before and after the injections is shown in Table I, where an increase in the NA output can be seen both during the first and the second collection period following the injection. No definite relationship between dose of acetylcholine and catechol amine excretion was observed.

After prostigmine administration an increased catechol amine excretion was also noted. The effect was quite similar to that observed for the highest dose of acetylcholine. It is to be noted that prostigmine did not produce similar "muscarinic" effects as acetylcholine in the dose used.

Table II shows the average catechol amine excretion in all 21 experiments before and after administration of acetylcholine. The average excretion was also measured during 10 different days in rats receiving no treatment or saline only. The same rats were also treated with prostigmine during 4 different days. The mean differences and S. E. in Table II are calculated on the basis of individual group differences. After injection of acetylcholine a 64 % increase of NA is observed (from 97 to 159 ng/kg/hr) in contrast to an average 12 % increase in the untreated animals. An 80 %

Table I.

The effect of acetylcholine and prostigmine on catechol amine excretion in rats (in ng/kg/hr).

Treatment Dose per rat	No. of series	Before injection		After injection			
				0—2 hours		2—4 hours	
		NA	A	NA	A	NA	A
None	6	119 (101—158)	3.0 (0—8.6)	112 (57—210)	3.6 (0—13.8)	131 (100—210)	3.4 (0—10.0)
Saline 0.9 % 0.2 ml	4	106 (84—125)	3.5 (0—13.5)	125 (108—140)	5.2 (0—18.8)	137 (118—170)	5.2 (0—11.1)
Acetylcholine 0.05 mg	4	114 (101—128)	14.8 (6.4—23.5)	151 (142—168)	9.7 (3—18.2)	162 (148—182)	6.4 (2.8—14.8)
» 0.1 mg	4	97 (47—149)	13.2 (9.7—17.5)	174 (114—270)	13.5 (0—28.5)	170 (123—320)	4.6 (0—8.5).
» 0.2 mg	5	91 (49—122)	8.4 (0—21.5)	152 (79—182)	10.0 (0—28.5)	158 (57—200)	5.8 (0—16.4)
» 0.1 mg	4	74 (51—105)	3.7 (0—10.5)	157 (118—200)	7.0 (0—15.8)	143 (96—255)	8.5 (0—26.0)
» 1 mg	4	107 (94—170)	7.9 (0—18.8)	181 (122—328)	21.7 (0—54.0)	264 (140—510)	15.7 (0—44.0)
Prostigmine 5 μg	4	121 (87—177)	6.3 (0—14.0)	171 (125—200)	22.5 (15.0 55.0)	219 (135—280)	13.3 (9.9—18.0)

Table II.

Effect of acetylcholine and prostigmine on mean catechol amine excretion in ng/kg/hr.

Treatment	No. of series	Before injection I		After injection 0—2 hrs II		2—4 hrs III	
		NA	A	NA	A	NA	A
Untreated or saline ...	10	114	3.2	128	4.2	141	4.1
Acetylcholine 0.05—1 mg per rat	21	97	10	159	12.3	175	8.1
Prostigmine 5 μg per rat	4	121	6.3	171	22.5	219	13.3

Diff.	II — I		III — I	
	NA	A	NA	A
Untreated or saline ...	14±11	1.0 ± 1.7	27± 9.8	0.9 ± 1.5
Acetylcholine 0.05—1 mg per rat	62±11.3 ***	2.3 ± 3.6	78±18.9***	—1.9 ± 1.9
Prostigmine 5 μg per rat	50±22 (*)	16.2±14	98±33**	7.0 ± 4.1

S. E. calculated on differences in individual groups.
P = 0.05—0.01 * probably significant
P = 0.01—0.001 ** significant
P = <0.001 *** highly significant

increase of NA is also observed during the third collection in contrast to a 24 % spontaneous increase in the untreated animals. As to the adrenaline excretion no significant differences were observed after varying doses of acetylcholine but it should be noted that the control values varied considerably (range 0—23.5 ng/kg/hr). Occasional high adrenaline values were found after the biggest doses of acetylcholine. After administration of prostigmine adrenaline excretion was also strongly increased in some cases.

When the excretion figures from the second and third collection are compared it is observed that noradrenaline tends to increase during the day, while adrenaline tends to decrease.

Comment.

The present experiments have demonstrated a highly significant rise in the NA excretion after administration of acetylcholine in the dose range 0.05—1 mg to rats. Even if the diurnal increase

in NA excretion is taken into account the increase is significant (48 ± 15.8 ng/kg/hr).

The figures indicate that there is, on the whole, no clear increase in adrenaline excretion. In two of the series receiving 1 mg acetylcholine there was a marked increase in the adrenaline excretion, however (29.5 and 54 ng/kg/hr respectively).

Since it would be expected that stimulation of the suprarenals should result in an increased secretion of chiefly adrenaline in the rat, it appears likely that the augmented output of NA originates from extra-medullary sources, *i. e.* sympathetic nerves. Whether this effect is induced centrally, reflexly, or peripherally cannot be decided from the present experiments. The possibility that acetylcholine should exert a central stimulating action leading solely to activation of the noradrenaline producing cells of the suprarenal medulla (HILLARP and HÖKFELT 1953, FOLKOW and EULER 1954) does not seem very probable.

The irregular shape of the dose-response curve is probably due to variations in the resorption and inactivation of acetylcholine.

The effect of prostigmine showed wide variations. In one series the adrenaline output was very high (average 55 ng/kg/hr) which was comparable to the highest value observed after 1 mg acetylcholine. A clear tendency to an increase in the NA excretion was noted both during the first and the second post-injection period (probably significant). Prostigmine thus produced an effect of essentially the same kind as acetylcholine.

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A Study of Central Regulation of Rumination and Reticulo-Ruminal Motility.

By

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Received 29 January 1959.

Abstract.

ANDERSSON, B., R. L. KITCHELL and N. PERSSON. A study of central regulation of rumination and reticulo-ruminal motility. *Acta physiol. scand.* 1959. *46*. 319—338. — Rumination, emesis and increased reticulo-ruminal motility have been produced by electrical stimulation at different sites in the medulla oblongata of conscious goats. In the region of the dorsal motor nucleus of the vagus, stimulations among other effects caused arrest of reticulo-ruminal motility, followed by abnormally increased motility after cessation of stimulation.

Electrical stimulation has previously been used to localize different medullary "centres". All this work has, however, been performed in anaesthetized animals or in decerebrate preparations and the stimuli applied have been very strong.

With the HESS' technique (1932, 1949) it is possible to stimulate sites in the medulla oblongata of the conscious goat (ANDERSSON 1951). The following study was undertaken to find out whether

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or not systematically performed, discretely localized stimulations of different areas of the medulla oblongata of the conscious goat could localize a "reticulo-ruminal centre" (IGGO 1951) more distinctly than previous work. It was also hoped that the results would add to our knowledge about the central regulation of rumination.

Methods.

Seventeen adult female goats were used for the experiments. Closed ruminal, abomasal and (in five of the animals) reticular fistulae were placed in the animals about one month earlier in a similar manner as described previously (ANDERSSON, KITCHELL and PERSSON 1958).

Stimulation technique: HESS' technique (1932, 1949) was used with some modifications. To facilitate orientation of the electrodes in the brain stem, a day or two before the experiment the tips of two sewing-needles were fixed about 1 cm apart in the skull as close to the dorsal midline as possible and X-ray pictures were taken of the head from the side and from above.

The operation for placing the electrodes in position was performed under local anaesthesia. A dorsal midline incision was made through the skin over the posterior part of the skull thus exposing the tips of the sewing-needles. The correct placement of the holder (Fig. 1 A) for guidance and fixation of the electrodes was thus facilitated by the X-ray pictures showing the position of the tips of the sewing-needles in two planes in combination with the direct viewing of these tips on the surface of the exposed bone of the skull.

Three parallel electrodes (0.3 mm in diameter) with uninsulated tips having a length of 0.2 mm were simultaneously placed in the brain stem at a distance of 2 mm from each other (Fig. 1 B). In order to reach the caudal part of the medulla oblongata with the electrodes the holder (A) had to be fixed in a slanting position on the skull by help of a rod (C) attached to the posterior part of the holder. The electrodes, which were fixed to the guiding rods (D) by screw-nuts (E), could be lowered step by step by screwing the nuts downwards a given distance.

During the experiments the animals were either moving freely in a pen or, when stomach motility was being recorded, they were placed in a stall and permitted free access to food and water.

Stimulus: Unipolar stimulation only was used. The resistance with the electrodes in situ varied between 5 and 10 kΩ. Damped square wave pulses with the DC-component removed were used as stimuli. Duration of pulses was 1 msec. Other parameters of stimulation were 0.01—0.03 mA and 10—50 cps.

Recording techniques: Stomach motility and chewing activity were recorded as described previously (ANDERSSON *et al.* 1958). During experiments in which the diuresis of the animals was followed the

Fig. 1. A paramedian sagittal section through the cranium of a goat with electrodes fixed in the manner used for medullary stimulation.

A: Electrode holder screwed on to the skull.
B: Uninsulated tips of the electrodes.
C: A rod fixed on to the caudal part of the holder giving the holder a slanting position on the skull.
D: Threaded guiding rods for the electrodes.
E: Screw-nuts to fix the electrodes on the guiding rods.
F: A contact for connection of the electrodes with the wires from the stimulator. The contact was during the experiment fixed on to one of the horns of the animal.

procedure was the same as that described earlier (ANDERSSON and PERSSON 1958). Experiments in which rumination or emesis was obtained as effects were partly filmed.

Histological technique: After an experiment was finished the animal was sacrificed with nembutal intravenously and decapitated. The head was perfused first with Ringer's solution and then with Bodian's (no. 3)

fluid. The posterior part of the brain stem with the cerebellum attached to it was embedded in celloidin and cut in serial transverse sections 50 μ thick. The plane of sectioning was directed as close as possible parallel to the electrode tracks. Alternate sections were stained with toluidin blue (Nissl) and haematoxylin (Loyez).

Results.

From the very beginning of this series of experiments it became apparent that very weak stimuli had to be used in order to get distinct and clear cut results. Stimuli of a strength which in the goat might be used with advantage for hypothalamic stimulation (0.1—0.3 mA, 50 cps., pulse duration 3 msec) at most sites in the medulla oblongata caused a complex pattern of strong effects, *i. e.* pain, motoric responses and complete arrest of respiration. It was obviously of little value to try to study alimentary effects under such circumstances. It was therefore decided to make all exploratory stimulations with a very weak stimulus (0.01 mA).

It is outside the aim of this study to give a detailed report of respiratory effects obtained. In general it can be stated that although many points of stimulation were situated within the expiratory and inspiratory "centres", weak stimuli at sites in the caudal part of these "centres" were never seen to cause continuous arrest of respiration during either expiration or inspiration. Rostrally in the explored part of the medulla oblongata stimulations within the central part of the formatio reticularis grisea were seen to cause a short initial arrest of respiration followed by slow respiration as long as the stimulation was continued (Fig. 2, A, B and C). Temporary arrest of respiration occurred at sites where stimulation caused emesis or rumination. The arrest appeared only during retching and expulsion of vomitus or during regurgitation.

Vomiting and retching as effects of medullary stimulation.

Emesis occurs rarely in ruminants and it might not be justified to compare the act of vomition in a ruminant animal with vomition in an animal with a simple stomach like the cat or the dog. Nevertheless, stimulations close to the wall of the IVth ventricle within a narrow region between the area postrema and the caudal

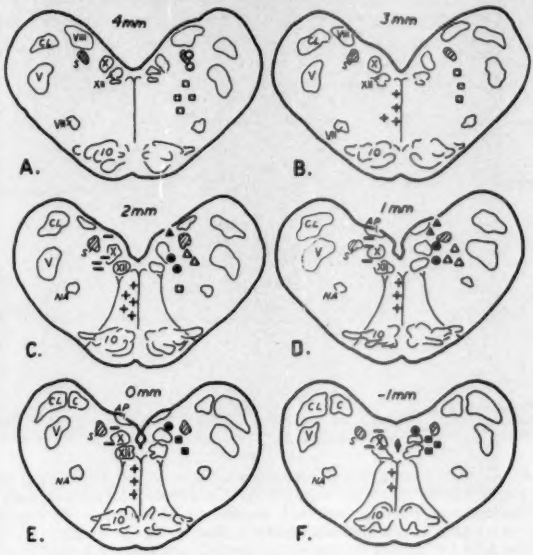

Fig. 2. Projection drawings of the medulla oblongata of the goat. The levels of the sections are indicated in mm with reference to the obex as zero. Plotted on the drawings are symbols indicating the effects of electrical stimulation.

- ●: Rumination.
- ▲: Emesis.
- ■: Coughing.
- ○: Rhythmic mastication.
- △: Retching with occasional expulsions of vomitus.
- □: Temporary arrest of respiration followed by slow respiration.
- +: Increased reticulo-ruminal motility.
- −: Arrest of reticulo-ruminal motility followed by abnormally increased motility on cessation of stimulation.

AP: Area postrema. C: Cuneate nucleus.
CL: Lateral cuneate nucleus. IO: Inferior olivary nucleus.
NA: Nucleus ambigus. S: Tractus solitarius.
V: Spinal nucleus of the trigeminal nerve.
VII: Facial nucleus. VIII: Medial vestibular nucleus.
X: Dorsal motor nucleus of the vagus.
XII: Hypoglossal nucleus.

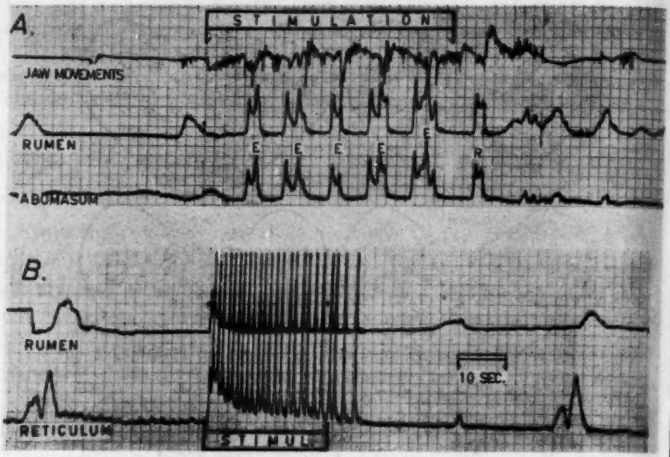

Fig. 3. *A:* An electromyogram of the masseter muscle (upper tracing) and simultaneous recordings of pressure changes in the rumen (dorsal sac) and the abomasum. Stimulation causing repeated expulsions of vomitus (E) was made at a site in the medulla shown in Fig. 4. R: Retching.

Horizontal axis, 5 divisions: 10 sec.
Vertical axis, 1 division: Rumen 25 mm H_2O
Abomasum 30 mm H_2O.

B: Similar recordings of pressure changes in the rumen and the reticulum during a period when coughing was induced by medullary stimulation.

Horizontal axis, 5 divisions: 10 sec.
Vertical axis, 1 division: Rumen 25 mm H_2O
Reticulum 20 mm H_2O.

pole of the medial vestibular nucleus (Fig. 2 C and D) were seen to produce an effect in the goats very similar to the act of vomition in the dog and the cat. The goats lowered their head and after a rapid inspiration tried to expire with the epiglottis closed and increased the intraabdominal pressure by strong contraction of the abdominal muscles, all of which resulted in an expulsion of some of the reticulo-ruminal contents. The vomitus either came out of the mouth and dropped in front of the animals or was reswallowed. At this site of stimulation vomiting appeared without prior retching. Continuous stimulation caused repeated expulsions of vomitus at intervals of about 10 seconds. Fig. 3 A shows a record of repeated expulsions of vomitus obtained during stimula-

Fig. 4. Parts of a transverse section through the medulla oblongata of a goat at a level 2 mm rostral to the obex. Lateral to the rostral pole of the area postrema an electrocoagulation (E) can be seen, which was made at the site where electrical stimulation had the emetic effect recorded in Fig. 3 A.

AP: The rostral pole of the area postrema.
PC: Choroid plexus of the 4th ventricle.
S: Traetus solitarius.
4: The 4th ventricle.
X: Dorsal motor nucleus of the vagus.
XII: Hypoglossal nucleus.

Stain: toluidin blue (Nissl). × 20.

tion at a site indicated by the electrocoagulation seen in Fig. 4. Retching together with occasional expulsions of vomitus was seen during stimulation within the formatio reticularis grisea ventral and lateral to the tractus solitarius (Fig. 2 C and D). The area corresponds roughly to the part of the reticular formation in which electrical stimulation causes vomiting in decerebrate cats (Borison and Wang 1949; Wang and Borison 1950).

The spontaneous expulsion of vomitus in simple stomached animals is associated with a firm contraction of the pyloric part of the stomach (Hatcher 1924). The method of recording contractions of the abomasum during the present study made it difficult

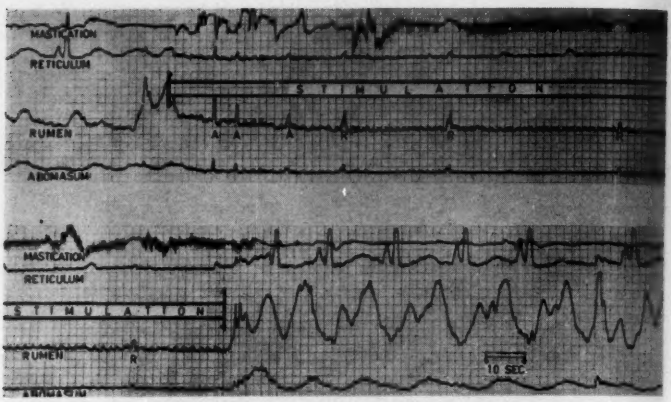

Fig. 5. An electromyogram of the masseter muscle (upper tracing) and simultaneous recordings of pressure changes in the reticulum, the rumen (dorsal sac) and the abomasum. Continuous sets of records. Medullary stimulation at a site shown in Fig. 6 is seen to have caused arrest of reticulo-ruminal motility and rumination followed by abnormally increased reticulo-ruminal motility after cessation of stimulation.

A: Attempts by the goat to regurgitate.
R: Regurgitation.

Horizontal axis, 5 divisions: 10 sec.
Vertical axis, 1 division: Reticulum 40 mm H_2O
Rumen 20 mm H_2O
Abomasum 40 mm H_2O.

to judge to what degree the expulsions of vomitus were associated with a contraction of the pyloric part of the abomasum. The strong increase in the intraabdominal pressure masked the activity of the abomasum. No augmented abomasal motility was however observed upon cessation of stimulation.

As apomorphine and copper sulfate emesis in the dog (ANDERSSON and LARSSON 1954) causes a release of antidiuretic hormone from the neurohypophysis, it seemed to be of interest to study whether stimulations producing emesis in the goat had the same effect. In two animals tested after hydration a stimulation which caused emesis also produced a marked inhibition of water diuresis of neurohypophyseal type. In both cases were the sites of stimulation located just lateral to the area postrema.

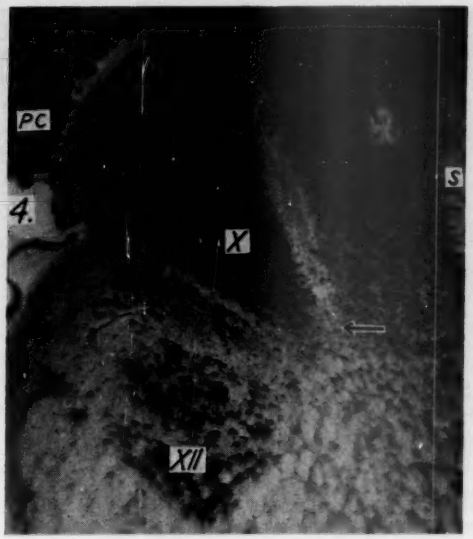

Fig. 6. Parts of a transverse section through the medulla oblongata of a goat at a level 1.5 mm rostral to the obex. An electrode track and (indicated by the arrow at its ventral end) the site can be seen at which stimulation had the effect recorded in Fig. 5, namely rumination and arrest of reticulo-ruminal motility followed by abnormally increased motility of the forestomach after cessation of stimulation.

PC: Choroid plexus of the 4th ventricle.
S: Tractus solitarius.
4: The 4th ventricle.
X: Dorsal motor nucleus of the vagus.
XII: Hypoglossal nucleus.

Stain: toluidin blue (Nissl). × 20.

Coughing.

Coughing as a result of stimulation was seen when loci posterior and medial to the "retching area" were stimulated (Fig. 2 E and F). Coughing appeared repeatedly during the entire periods of stimulation and for 5 to 10 seconds afterwards. It had a much higher frequency than the retching and the expulsions of vomitus (Fig. 3 B).

Mastication.

Stimulations somewhat rostral to the "retching area" adjacent to the tractus solitarius (Fig. 2 A) produced rhythmic masticatory

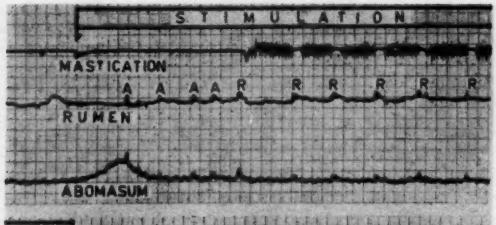

Fig. 7. An electromyogram of the masseter muscle (upper tracing) and simultaneous recordings of pressure changes in the rumen (dorsal sac) and the abomasum. Continuous sets of records. Rumination with abnormally high frequency of regurgitation was obtained by stimulation in the caudal part of the "rumination area" of the medulla oblongata.

A: Attempts by the goat to regurgitate.
R: Regurgitation.

Horizontal axis, 5 divisions: 10 sec.
Vertical axis, 1 division: Rumen 40 mm H_2O
Abomasum 30 mm H_2O.

movements which were sometimes periodical and similar to the remastication seen during rumination. No signs of rejection and licking of the type observed during stimulation of the thalamic "relay" for taste in the goat (ANDERSSON and JEWELL 1957) were seen in combination with these masticatory movements.

Rumination combined with temporary arrest of reticulo-ruminal motility.

Rumination, outwardly indistinguishable from spontaneous rumination, was obtained as a result of stimulation of an area adjacent to the "retching area" located medial to the tractus solitarius between it and the dorsal motor nucleus of the vagus and the hypoglossal nucleus (Fig. 2 C and D). After a few attempts by the goats to ruminate, indicated by a slight but rapid increase in the intraabdominal pressure, regurgitation and subsequent

remastication occurred followed by swallowing of the remasticated bolus. Rumination continued with normal length of the cycles during the entire period of stimulation. Fig. 5 shows a record of rumination obtained during stimulation at a site visible in Fig. 6. Rumination with an abnormally high frequency of regurgitation (Fig. 7) was obtained during stimulation slightly more caudally in the medulla at the level of the obex and about 1 mm caudal to it. The two effective sites of stimulation were located just dorsal to the dorsal vagal nucleus (Fig. 2 E and F).

At sites where stimulation resulted in rumination, the stimulus strength could be increased three times and still produced only rumination. Records of reticulo-ruminal motility, however, did reveal a striking difference between spontaneous and experimentally induced rumination. During the entire period of stimulation there was a complete arrest of reticular and ruminal contraction. After cessation of stimulation an abnormally rapid reticulo-ruminal motility started immediately (Fig. 5). The frequency of reticular contractions went up as much as to 4 to 5 per minute. The increase in reticulo-ruminal motility persisted for about 5 minutes after a two minutes' arrest during one period of stimulation. This same phenomenon was also observed due to stimulation at some sites at which rumination was not obtained as effect. Also these sites were, however, located in the vicinity of the dorsal vagal nucleus. The region in which stimulation caused arrest of reticular and ruminal motility followed by abnormally increased motility is indicated by negative signs in Fig. 2 (C, D, E and F).

Increased reticulo-ruminal motility as a result of medullary stimulation.

In view of earlier findings in the anaesthetized sheep (Bell and Lawn 1955) it was unexpected to find that electrical stimulation within the formatio reticularis grisea of the conscious goat did not cause direct augmentation of reticulo-ruminal motility. If increased motility of the forestomach was observed, it occurred on cessation of stimulation (see above).

Stimulations within the formatio reticularis alba, *i. e.* the area medial to the root filaments of the hypoglossal nerve and dorsal to the inferior olivary nucleus, did, however, cause a marked augmentation of reticulo-ruminal motility (Fig. 2, plus-signs). Fig. 8

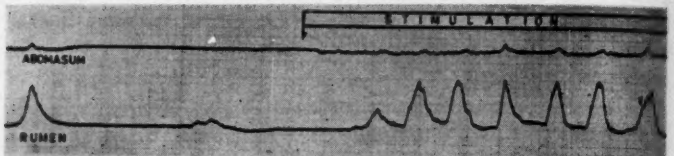

Fig. 8. Continuous recordings of pressure changes in the abomasum and the rumen (dorsal sac). Increased ruminal motility is seen during stimulation medially in the formatio reticularis alba.

Horizontal axis, 5 divisions: 10 sec.
Vertical axis, 1 division: Abomasum 30 mm H_2O
Rumen 30 mm H_2O.

shows increased ruminal motility caused by stimulation within this area of the medulla oblongata. Most pronounced augmentation of forestomach motility was obtained when stimulating at sites located near the midline close to the raphe. Within 10 to 20 sec after cessation of stimulation, the motility of the reticulum and rumen was back to prestimulation frequency. If the period of stimulation was for a minute or longer the goat might start to eat or, when the rumen was full, to ruminate. The increased appetite and rumination, which were obtained at the same site of stimulation but on different occasions, were obviously not primary effects of stimulation as they appeared a considerable time after the augmentation of the reticulo-ruminal motility. Intravenous injection of adrenaline (10 μg/kg b.wt.) or noradrenaline (8 μg/kg b. wt.) blocked the reticulo-ruminal response to stimulation for a period of about five minutes. It then gradually reappeared.

Amoroso, Bell and Rosenberg (1954) have found that stimulation within this part of the medulla oblongata of the anaesthetized sheep causes arrest of respiration during inspiration and produces a fall of blood pressure. No effects on respiration were observed in the conscious goat. The stimulations which caused

marked augmentation of reticulo-ruminal motility did not affect the heart rate as measured by electrocardiograms taken before, during and after the period of stimulation.

The response of the goats to application of copper sulfate in the abomasum.

On the basis of experiments showing that large doses of apomorphine cause hyperkinesis of the rumen and forced mastication in the sheep, ARDUINI and DAGNINO (1953) have suggested that rumination is a mechanism corresponding to emesis in animals with a simple stomach and that a postulated "rumination centre" would thus be identical to the vomiting "centre" in other mammals. Varying doses of apomorphine were however not seen to cause rumination in the goat (ANDERSSON *et al.* 1958).

During the present study it became apparent that emesis, as well as rumination, could be elicited by electrical stimulation in the medulla oblongata and that in some cases there was only a slight spatial difference between two points producing the two effects. It seemed therefore to be of interest to study the effect of an emetic, like copper sulfate, which produces its results by acting mainly peripherally. Towards the end of each experiment in six of the animals, a solution of 10 % copper sulfate in doses from 100—125 mg per kg b.wt. was injected through the fistula into the abomasum. In two of the animals the presence of copper sulfate in the abomasum caused marked augmentation of abomasal motility and after several minutes repeated expulsions of vomitus occurred which were of the same character as those obtained when sites just lateral to the area postrema were stimulated. In the remaining four animals the effect on abomasal motility was much less pronounced and after a latency of 5 to 15 min these goats started to ruminate.

Discussion.

It is evident from the results reported here that rumination can be elicited by electrical stimulation within a fairly small area of the medulla oblongata in the goat. This area is located medial to the tractus solitarius between it and the dorsal vagal motor nucleus and the hypoglossal nucleus and extends about 2 mm

rostral to the obex and 1 mm caudal to it (Fig. 2, C, D, E and F). Evidence is presented that this part of the brain stem might be a locus for the central coordination of all mechanisms involved in the process of rumination. Although the "rumination area" was found to be anatomically closely related to the part of the medulla in which stimulations caused emesis and retching, several features of this study indicate that the central coordinating mechanisms for rumination and for emesis are not identical. Thus at a site of stimulation where a weak stimulus had been found to produce rumination, the stimulus strength could be increased three-fold and the effect still remained to be rumination. Lowering the strength of stimulation at a site where emesis was obtained as the effect, only reduced the emetic response but the response was never seen to be converted into rumination. If emesis in a ruminant animal would be the manifestation of a strong activation of a "rumination centre" a functional overlapping of the two effects ought to have been found at the same site of stimulation. The different nature of the two responses also indicates that two different coordinating mechanisms are involved. Thus during rumination regurgitation caused very little increase of intra-abdominal pressure and at most sites of stimulation regurgitation occurred with a frequency of one in 30—50 seconds, *i. e.* with the same frequency as is seen during spontaneous rumination (Fig. 5). The expulsions of vomitus on the other hand appeared with a higher frequency and involved a very marked increase of intra-abdominal pressure (Fig. 3 A). The fact that presence of copper sulfate in the abomasum in two animals led to emesis but in the others resulted in rumination must not necessarily be taken as a support for the view that one and the same "centre" coordinates the two mechanisms. The varying response might well depend on individual differences in the reactivity of one or the other of two separate "centres".

Using different methods to approach the problem BORISON and WANG have in a long series of experiments succeeded in elucidating the central coordinating mechanism for emesis. A comprehensive review of this work has been given (BORISON and WANG 1953). This work has made evident that a "vomiting centre" is located in the formatio reticularis grisea adjacent to and ventrolateral to the tractus solitarius. Centrally acting emetics do not stimulate this "centre" directly but by the intermediation of structures located medially in the medulla oblongata close to the

ventricular wall ("chemoreceptor trigger zone"). Bilateral destruction of the area postrema thus abolishes the emetic action of apomorphine in the dog, although peripherally acting emetics like copper sulfate still activate the vomiting "centre" reflexly. WANG and BORISON (1950) did not find it possible to elicit vomiting by direct electrical stimulation of the area postrema or its close vicinity in decerebrate cats. Stimulations just lateral to the area postrema did however produce emesis in the conscious goat, and the response was more like the act of vomition in an animal with a simple stomach than was the response to stimulation of an area of the formatio reticularis corresponding to the vomiting "centre" of BORISON and WANG. Stimulations within the latter area produced mainly retching and only occasionally real expulsion of vomitus. It might be expected that the direct stimulation of the "chemoreceptor trigger zone" should elicit not only the act of vomition but also nausea, indicated by salivation, and other prodromal signs which are seen following the application of centrally acting emetics. It is perhaps significant that in one of the protocols from the experiments, in which the lateral margin of the area postrema later was found to have been stimulated, is noted that "the animal looks nauseated during stimulation".

The action of the cholinergic vagal efferents is not essential for spontaneous vomiting (BROOKS and LUCKHARDT 1915) or for vomiting elicited by electrical stimulation of the vomiting "centre" (BORISON and WANG 1949). This is also true regarding spontaneous rumination (WESTER 1923) and rumination elicited by stimulation of the teats in lactating animals (ANDERSSON *et al.* 1958). Further evidence that reticular and ruminal motility is not needed during rumination may be seen by observing the effects of medullary stimulation which elicited rumination and noting that reticular and ruminal motility was inhibited during such stimulations.

Rhythmic mastication similar in nature to movements observed during remastication was observed when stimulation was performed at sites close to the tractus solitarius rostral to the "rumination area" (Fig. 2 A). The effect might be due to direct stimulation of parts of the nucleus of the tractus solitarius or the stimulation of afferent fibres running to it which normally are activated by the presence of regurgitated material in the mouth during the rumination cycle.

The present study has not directly demonstrated the localization of the medullary "pace-maker" for reticulo-ruminal motility.

IGGO (1956) has shown that sheep decerebrated in the intercollicular plane through the midbrain may retain spontaneous, rhythmic reticulo-ruminal contractions. It is also possible in such a preparation to initiate rhythmic contractions by central vagal stimulation. This is even possible after section of the brain stem as far posterior as 5 mm caudal to the ponto-medullary junction (DUSSARDIER and ALBE-FESSARD 1954). DZUIK and SELLERS (1955) have further shown that the intrathoracic stimulation of the dorsal and ventral vagi in the conscious calf increases the rhythmic activity in the forestomach. The effect is probably due to the activation of a medullary "centre" by the way of vagal afferents, since stimulation of only the peripheral end of the vagus seems to cause persistent, increased tonus in reticulum and rumen and does not produce the rhythmic activity seen in the intact animal (MARSCHALL 1910; POPOW, KUDRJAVCEW and KRASOUSKY 1933). The normal pattern of reticulo-ruminal motility thus seems to be dependent upon a rhythmically working "centre" in the brain. Since distension of the abomasum inhibits reticulo-ruminal motility (PHILLIPSON 1939) it is evident that not only stimulatory but also inhibitory impulses reach this "centre" from the stomach. The inhibition of reticular and ruminal motility observed when sites in the vicinity of the dorsal vagal nucleus were stimulated was obviously also due to an inhibition of the "centre" regulating the motility of the forestomach, since vigorous and frequent contractions in the reticulum and rumen appeared immediately after cessation of stimulation. The latter is not likely to have happened if the inhibition would have been due to sympathetic discharge causing peripheral inhibition of stomach motility. IGGO (1956) found that when the gastric afferent fibres in the vagi were stimulated electrically in decerebrate sheep, two reticular contractions could not be elicited with less than a 10 sec interval between them. If this frequency represents the effect of maximal discharge from the reticulo-ruminal "centre" it would mean that cessation of stimulation in the "rumination area" led to almost maximal activity in the reticulo-ruminal "centre" (4 to 5 reticular contractions in one minute) (Fig. 5). It might be assumed that the "centre" for control of reticulo-ruminal motility is located in close anatomical relation to the area where stimulations caused arrest of this motility. Much evidence based upon anatomical (MOLHANT 1910, GETZ and SIRNES 1949, KITCHELL, STROMBERG and DAVIS 1956) and physiological (LAUGHTON 1929) work suggests that a gastric

"centre" is located in the dorsal motor nucleus of the vagus. The present study speaks in favour of the dorsal motor nucleus of the vagus being the "pace-maker" for reticulo-ruminal motility. Since one of the inhibitory points was situated in the very lateral part of this nucleus (Fig. 6) and others close to its lateral border there is reason to believe that a continuous electrical stimulation close to or in the dorsal motor nucleus of the vagus might block a normally occurring rhythmic activity in this nucleus, resulting in arrest of reticulo-ruminal motility. If this assumption holds true it would further indicate that there is a close interaction between the dorsal vagal nucleus of both sides of the medulla oblongata since a unilateral stimulation caused complete inhibition of reticulo-ruminal motility. A rebound phenomenon would then be the most likely explanation of the hyperactivity seen on cessation of stimulation.

Since, however, at some sites of stimulation, located at some distance from the dorsal vagal nucleus, this structure does not seem to have been directly affected by the stimulus it might also be assumed that the effect could partly have been due to a stimulation of inhibitory fibres reaching the nucleus.

The area of the medulla in which stimulations directly caused an increased, rhythmic reticulo-ruminal motility, *i. e.* the formatio reticularis alba (Plus-signs in Fig. 2), is occupied mainly by the medial lemniscus, the medial longitudinal fasciculus and internal arcuate fibres. Since rhythmic contractions of the forestomach appeared it is not likely that the effect was due to stimulation of vagal efferents. Most pronounced augmentation of reticulo-ruminal motility was obtained at sites of stimulation located medially close to the raphe. Sensory fibres of the second order, araising in the sensory nuclei of the cranial nerves (Ranson and Clark 1953), pass as internal arcuate fibres through the formatio reticularis alba, join the raphe and run along it for some distance before crossing over to the other side of the medulla. In a transverse section of the medulla oblongata of the goat in which the myelin has been stained, fibres can easily be traced passing from the raphe round the ventral margin of the hypoglossal nucleus. Some of these fibres disappear in the region of the dorsal motor nucleus of the vagus, some are seen to continue into the root filaments of the vagal nerve (Fig. 9). A possible explanation of the augmentation of reticulo-ruminal motility seen during stimulation within the formatio reticularis alba may thus be a stimulation of sensory

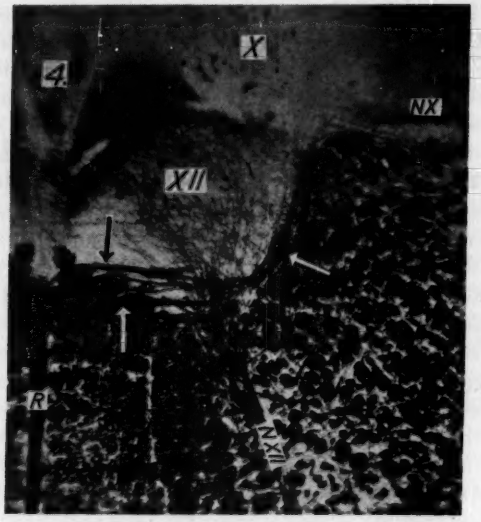

Fig. 9. Parts of a transverse section through the medulla oblongata of a goat at the level of the obex. Myelinated fibres are visible which pass from the raphe round the ventral margin of the hypoglossal nucleus through the root filaments of the hypoglossal nerve (indicated by the arrows). Some of these fibres disappear in the region of the dorsal motor nucleus of the vagus, some continue into root filaments of the vagal nerve.

R: Raphe.
4: The 4th ventricle.
X: Dorsal motor nucleus of the vagus.
XII: Hypoglossal nucleus.
N X: Root filaments of the vagal nerve.
N XII: Root filaments of the hypoglossal nerve.

Stain: haematoxylin (Loyez). × 20.

vagal fibres of the first and/or second order which in this region of the medulla cross over to the contralateral side. The density of such fibres might be expected to be greatest in the raphe.

The sensory stimulation of milking has been found to increase reticulo-ruminal motility in the lactating goat (ANDERSSON *et al.* 1958). Degeneration studies (HABEL 1956) do not exclude the possibility that afferent fibres from the stomach by means of the long pyloric, left ruminal and long abomasal nerves may reach the CNS by other routes than the vagi in ruminants. The reticulo-

ruminal "centre" may thus be stimulated by impulses which originate in the digestive tract and/or other parts of the body and reach the "centre" through other nerves than the vagi. Another possible explanation of the increased reticulo-ruminal motility observed during stimulation within the formatio reticularis alba is that it contains afferent fibres which transmit impulses of this kind.

Summary.

1. Electrical stimulation was performed at different sites in the caudal part of the medulla oblongata of conscious goats.

2. Stimulations within an area of the formatio reticularis grisea located ventral and lateral to the tractus solitarius produced retching and occasional vomiting. More vigorous vomiting without prior retching was seen due to stimulations at the lateral margin of the area postrema.

3. Stimulations medial to the "emetic area" between the tractus solitarius and the dorsal vagal and hypoglossal nuclei caused rumination. Several features of the study indicated that central coordinating mechanisms for rumination and for emesis are not identical.

4. Stimulations within or in the vicinity of the dorsal vagal nucleus caused a complete arrest of reticular and ruminal contractions, immediately followed by abnormally intense reticulo-ruminal motility after cessation of stimulation. This observation is taken as a support for the view that the dorsal vagal nucleus is the "pace-maker" for reticulo-ruminal motility.

5. Increased reticulo-ruminal motility as a direct effect of stimulation was only seen during stimulation at sites located within the formatio reticularis alba. Possible explanations of this phenomenon are discussed.

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On the Concentration of Bile Acids in the Human Intestine during Absorption.

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Abstract.

SJÖVALL, J. On the concentration of bile acids in the human intestine during absorption. *Acta physiol. scand.* 1959. *46*. 339—345. — The concentrations of glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acids have been determined in the intestinal contents of seven normal and one cholecystectomized subject during the absorption of a fluid test meal. Within 30 minutes after the meal the emptying of the gall-bladder caused a rise in total bile acid concentration to 13—46 meq/l, thereafter the concentration rapidly decreased, being between 2.5 and 10 meq/l during the main part of the period of digestion and absorption. Variations in the proportions of the bile acids were observed during this period but were usually small. The bile acid composition in the distal part of the small intestine seemed to be similar to that in the proximal part and the emptying of the gall-bladder caused no changes in bile acid concentration in the former part.

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In an investigation of the intestinal digestion and absorption in the human, the intestinal concentration of bile acids was one of the factors studied (BORGSTRÖM *et al.* 1957, where pertinent literature is quoted). The concentration of total bile acids during absorption of the test meal was reported but, since human bile contains several bile acids having somewhat different properties, it was thought to be of value to give a more complete account of the findings. This paper describes the relationships between the chief bile acids, *viz.* glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acids, at different levels of the intestine during the absorption of a test meal.

Experimental.

The material used for the experiments and the method for collection of intestinal contents have been described in the paper by BORGSTRÖM *et al.* (1957), where the composition of the test meal is also given.

Bile acids were determined by quantitative paper chromatography (SJÖVALL 1956) and a full description of this method has been submitted for publication in Clinica Chimica Acta. Glycocholic, taurocholic, glycochenodeoxycholic and glycodeoxycholic acids were determined separately whereas taurochenodeoxycholic and taurodeoxycholic acids were determined together. The intestinal content was directly used for paper chromatography without any preliminary purification and was stored at — 16° C until analyzed. Because of the large number of samples to be analyzed only single determinations of the bile acids were carried out in most instances.

Results and Discussion.

In all samples analyzed, spots were found on the chromatograms corresponding to glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acids. Since the taurine conjugates of the latter two acids were not separated in the quantitative determinations, qualitative separations with descending chromatography (SJÖVALL 1955) were performed in some cases and the presence of taurine conjugates of both acids was thus confirmed. This was also indicated by the sulphuric acid spectrum of the zone containing the mixture of taurochenodeoxycholic and taurodeoxycholic acids eluted from the paper chromatograms. Paper chromatography with phase systems suitable for the separation of free bile acids revealed traces of such acids only in a few

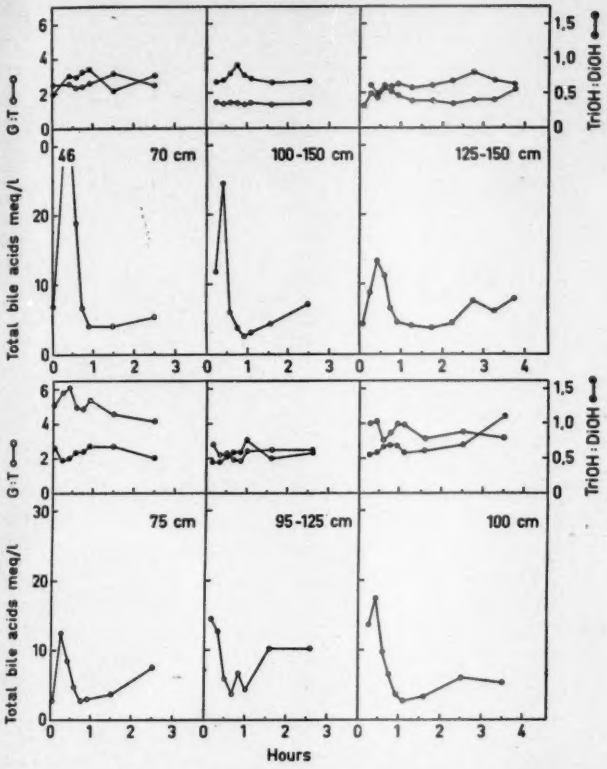

Fig. 1. Bile acid concentration and ratios between glycine and taurine conjugated bile acids (G:T) and between trihydroxy and dihydroxy bile acids (TriOH:DiOH) during the absorption of a test meal. All the subjects are healthy male medical students. The levels at which intestinal contents were collected are given as cm from the nose.

cases showing that practically all the bile acids occurred in the conjugated form. This was true also for two samples obtained from the distal part of the small intestine (about 250 cm from the nose).

The detailed findings on bile acid concentration and proportions between different bile acids after administration of a 500 ml test meal to six male medical students are illustrated in Fig. 1. During the first hour after the test meal 10-minute samples were

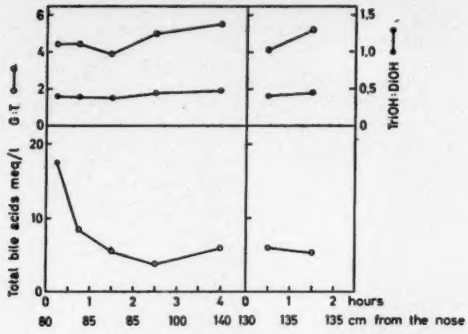

Fig. 2. Bile acid composition of intestinal contents simultaneously collected from two levels of the intestine during the digestion and absorption of a test meal. For abbreviations see Fig. 1.

taken and thereafter hourly samples were collected. The intestinal levels at which the samples were taken are indicated in the figure (cm, from the nose). In the cases where the tubing moved continually downwards, the distances from the nose at the beginning and the end of the experiments are given. For the significance of these values *cf.* HIRSCH, AHRENS and BLANKENHORN (1956), who found the distances from nose to pylorus, ligament of Treitz and the ileocecal valve to be around 65, 90 and 350 cm respectively. As seen from the figure the gallbladder empties within 30 min after the ingestion of the test meal. Usually the highest bile acid concentration is obtained between 15 and 30 min. The maximum concentration obtained is very variable which in the cases shown in Fig. 1 is probably due to differences in the concentration of the gallbladder bile and differences in the intestinal level at which the samples were taken. Probably as a result of dilution and absorption of the bile acids their concentration rapidly decreases and a minimum value is obtained between 45 min and 2 hours after the test meal. In contrast to the maximum value which varies between 13 and 46 meq/l the minimum value is fairly constant between 2.5 and 4.0 meq/l. The bile acid concentration then rises again to values between 5 and 10 meq/l, which are the approximative values found when a fasting individual is intubated and the contents from the duodenum and the upper part of the jejunum analyzed (SJÖVALL 1959).

Table I.

Bile acid composition in the distal part of the small intestine of a male subject.

No free bile acids were found.

Hours after test meal	Cm from the nose	Total bile acids meq/l	Ratio[1] GC:GCD:GD	Ratio[1] G:T
1—2	235	10.9	1.1:1:0.5	1.8
3—4	265	7.3	0.9:1:0.5	2.1

[1] Abbreviations: GC = glycocholic acid,
GCD = glycochenodeoxycholic acid,
GD = glycodeoxycholic acid,
G = glycine conjugates,
T = taurine conjugates.

As would be expected, the emptying of the gallbladder influences the bile acid concentration mostly at higher levels of the intestine. This is illustrated by the experiment shown in Fig. 2, where intestinal contents were collected from two intestinal levels at the same time after the administration of the test meal. Whereas a typical curve was obtained in the duodenum with a maximum concentration of 18 meq/l in the first half hour, the bile acid concentration at the level of 135 cm from the nose was 6.0 and 5.3 meq/l respectively, during the first and second hour after administration of the meal. Analyses of bile acids at still lower levels gave similar results, the lowest level to be investigated being about 250 cm from the nose (Table I).

The proportions between different bile acids are subject to large individual variations. The ratio between taurine and glycine conjugated bile acids was between 1.3 and 6.3 in the subjects investigated.

During the experimental period the ratio changed in several of the subjects, but these changes were individual and did not indicate a general preferential absorption of any one type of conjugate (Fig. 1 and 2).

Although in many cases changes were observed in the ratio between trihydroxy and dihydroxy bile acids during absorption, these changes were seldom very pronounced. The ratio between these acids was usually the same for the acids conjugated with taurine as for those conjugated with glycine. The proportions of

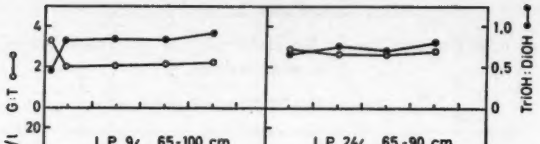

Fig. 3. Bile acid composition in the duodenal contents of a cholecystectomized patient during the absorption of a test meal.

cholic, chenodeoxycholic and deoxycholic acid as found by analyses of their glycine conjugates were similar to those found in the duodenal contents of fasting subjects (ENCRANTZ and SJÖVALL 1957), even at lower levels of the small intestine (Table I). During the emptying of the gallbladder the proportions of the acids sometimes changed a little, as was also sometimes the case when the polyvinyl tubing moved further down the intestine during the experimental period. These changes were individual and the percentage of any one bile acid seldom changed by more than 10 per cent.

Some experiments were also carried out with a cholecystectomized but otherwise healthy female subject. As seen in Fig. 3 the bile acid concentration as well as the proportions between the different bile acids in this subject did not differ from the corresponding values in the normal healthy subjects. Unfortunately the fasting values were not determined, so it cannot be decided whether the relatively high initial bile acid concentration was due to a release of bile from the bile ducts when the test meal was given or was just a high fasting value. Fasting values determined on other occasions in this subject were of the same order of magnitude as the initial values shown in Fig. 3.

As seen in Fig. 1 the gallbladder in normal subjects is emptied during the first 30 min after the test meal, giving rise to a high concentration of bile acids in the duodenum and upper part of jejunum. The concentration, however, rapidly decreases and since

most of the test meal is digested and absorbed after this decrease has taken place (BORGSTRÖM *et al.* 1957) it can be seen that the bile acid concentration during most of the period of absorption is between 2.5 and 10 meq/l even at lower levels of the intestine. The concentration of bile acids in the cholecystectomized subject is also within this range.

The changes in proportion between the different bile acids during absorption are usually small and probably without importance for digestion and absorption, since no differences in absorption were noted for the different subjects. That such changes may occur is, however, of importance when bile acid analyses of duodenal contents are used for the evaluation of the biliary bile acid composition.

The technical assistance of Mrs. ANN-MARIE ANDERSSON is gratefully acknowledged.

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Donated by Shipowner Anders Jahre.

The Influence of Temperature on the Electrocardiograms of Some Northern Reptiles.

By

KJELL JOHANSEN.

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Abstract.

JOHANSEN, K. The influence of temperature on the electrocardiograms of some northern reptiles. *Acta physiol. scand.* 1959. *46*. 346—357. — The relation between heart rate and body temperature in three species of Norwegian reptiles has been studied (Tripodonotus natrix L, Vipera berus L, Anguis fragilis L). In all species studied the heart beat *vs.* body temperature curves showed a logarithmic regression. The increments of heart frequency with temperature were found to be 5—6 beats/°C/minute inside a temperature interval from 8—32° C. The normal appearance of the electrocardiograms in the same three species of reptiles has been analyzed and the influence of temperature upon the waveform has been discussed.

The influence of temperature on the rate of various biological processes has been a stimulating research item for centuries and several basic questions have arisen. Do, for instance, biological phenomena vary by temperature according to wellknown chemical laws, or are other factors modifying the course of these processes? To what extent are the temperature coefficients for nervous and

humoral processes related to each other? In this connection the effects of temperature on the rate and activity of the intact heart has stimulated the present investigation.

According to paleonthologists, the modern reptiles, birds, and mammals have all developed from reptilian ancestors and the homeotherm mode of temperature regulation has likewise developed from the reptilian animal. It seems, therefore, likely that specialized, advanced reptiles of today, represented by snakes and lizards, are physiological research objects of special interest.

The Norwegian reptiles lend themselves specially to studies on the effects of temperature upon the heart activity. It is feasible to study the effects of temperature changes over a wide range without exceeding the temperature limits of normal physiological conditions in the animals. In mammals and birds, on the other hand, the normal temperature range has very narrow limits and therefore studies on intact homeotherms are scarce. Excised, denervated mammalian hearts have, however, been extensively studied concerning their relation to temperature (KNOWLTON and STARLING 1912). The intact hearts of hibernating and hypothermic mammals have also been studied extensively (DAWE *et al.* 1955, BIÖRCK and JOHANSSON 1955). Among the cold-blooded vertebrates, the hearts of fishes (CSINADY *et al.* 1940), and the hearts of amphibians (BARCROFT *et al.* 1930, TAYLOR 1930) have also been investigated. Intact reptilian hearts and especially those belonging to animals from the order Squamata, have, to the author's knowledge, not been subjected to such studies. Electrocardiograms from intact reptiles are rather scarce, and already BENEDICT (1932) advocated the necessity for more data in this field. In the present study the relation between heart rate and body temperature in three species of Norwegian reptiles is described, and electrocardiograms with waveform analysis related to body temperature are presented.

Material, Methods, and Experimental Procedure.

The material used for this study consists of three of the five species of reptiles existing in Norway. The investigation includes five specimens of the Norwegian viper (Vipera berus L), three common grass snakes (Tripodonotus natrix L), and eight legless lizards (Anguis fragilis L). All the animals seemed to be in good condition during the entire experi-

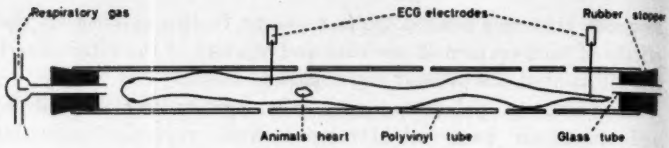

Fig. 1. The polyvinyl tube used for fixation of the animals during the recording of electrocardiograms.

mental periods. The body weight of the animals ranged between 80—120 g for the grass snakes, 60—90 g for the vipers, and 10—20 g for the lizards.

Fig. 1 illustrates how the experiments were arranged. The animal was placed head first into a flexible polyvinyl tube, which was closed in one end by a perforated rubber stopper. Once the animal was extended full length in the tube, the other end was closed by a rubber stopper similar to the first one. The inner diameter of the tube was slightly larger than the diameter of the snake to allow unrestricted respiratory movements. In this way the animal was well confined, and although some movements were possible in the sealed tube, these were not large enough to interfere with the experiments. Using this equipment it was possible to impose various environmental changes on the animals. In none of the experiments were the animals anesthetized, nor was any sort of sedatives administered. The registration of electrocardiograms has been done by means of needle electrodes. One electrode was placed near the heart in cranial position, while the other was placed in the caudal part of the animal. A Sanborn AC-DC preamplifier and a 4-channel heated stylus recorder were used. In those cases where the recording of body temperature of the animals was of importance, the experiments were performed in a specially temperature controlled room. The temperatures were recorded 2—4 cm into the cloaca by means of copper constantan thermocouples and a 12-channel continuously recording Brown Honeywell temperature recorder.

The experiments were usually started at an environmental temperature of about 15° C. The electrocardiographic electrodes were connected to the animal and a thermocouple fixed in the cloaca. The electrocardiogram and temperature recordings were started and registrations were obtained continuously throughout the experimental period. After a stabilizing period of about 5 min, the body temperature of the animal was changed gradually in steps of 1° C per 5 min, allowing the temperature to stabilize between each step. Usually the body temperature was at first increased to about 35° C and then lowered to 5—10° C. It was also tried to change the temperature in the opposite direction. Experiments carried out at different degrees of humidity in the environment did not change the heart rate/body temperature relations. Between the experiments, the animals were permitted to rest and the intervals between feeding were arranged so that the process of digestion should not interfere with the experiments.

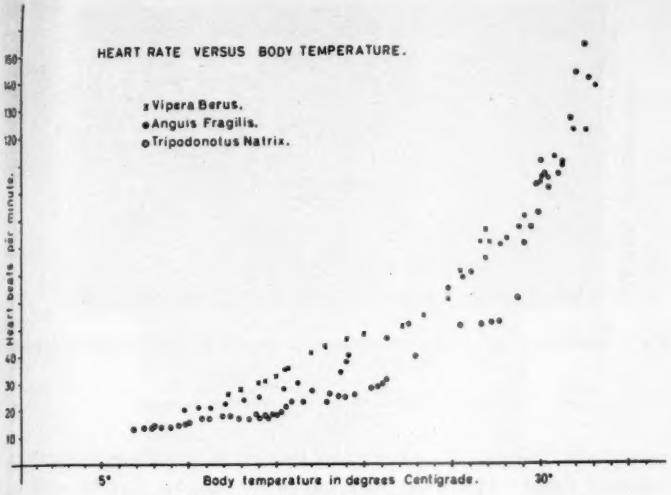

Fig. 2. Diagram showing the relation between heart frequency and body temperature in all species studied. Note the exponential regression of the curve.

Results.

The relation between heart rate and body temperature.

A high correlation between heart rate and body temperature was found for the animals investigated. This refers to subsequent experiments on the same species, as well as to compared results for all species investigated.

The experiments were performed during the months April to November, and no evidence of seasonal variations in the heart rate/body temperature curve was observed in this period. Meanwhile, it seemed evident that within the temperature range from 32° C and upwards, normal values of body temperature were surpassed in these animals. In this temperature range, the results were often inconsistent and there was a certain tendency to a decrease in heart rate. These results, however, apparently do not apply to normal physiological conditions of the animals and are consequently not plotted into the curves. At the lower body temperature limit, there were no signs of escape reactions or discomfort of the animals. As seen in Fig. 2 the relation between

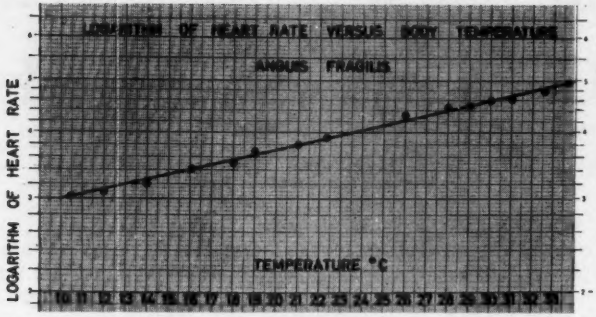

Fig. 3. Heart rate versus body temperature in Anguis fragilis plotted on a semi-logarithmic scale.

heart rate and body temperature showed a tendency towards a concave shape. The heart rate curve for Anguis fragilis was in particular found to be of the exponential type. See Fig. 3, where these data are plotted on a semi-logarithmic scale.

Electrocardiographic findings.

Normal electrocardiograms from a grass-snake and a viper and the changes recorded at different body temperatures are presented in Figs. 4 and 5.

The P-wave.

The P-wave was always easy to identify in Vipera berus and Tripodonotus natrix. In Anguis fragilis it was more obscure. Tripodonotus natrix always showed a negative P-wave together with a positive T-wave, while in Vipera berus, a positive P-wave always was found. With increasing body temperature and heart rate, the P-wave duration became shorter.

The duration of the P-wave in Vipera berus ranged between 0.05 sec at b. t. 12.3° C, and 0.01 sec at b. t. 26.9° C. The same values in Tripodonotus natrix were 0.10 sec at b. t. 7.2° C, and 0.015 sec at b. t. 32.4° C. The amplitudes did not change appreciably, although a slight elevation could be detected at the lower temperatures.

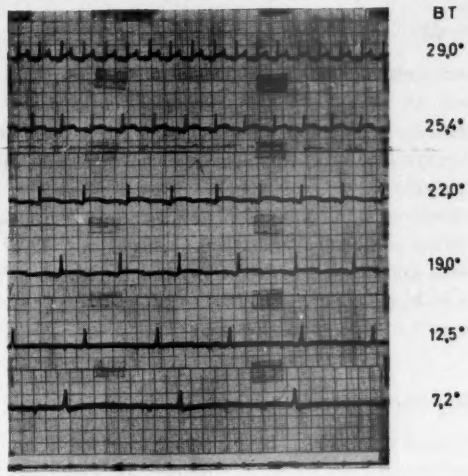

Fig. 4. Electrocardiograms recorded at different body temperatures in the common grass-snake, Tripodonotus natrix.

Time marks: 1 sec.

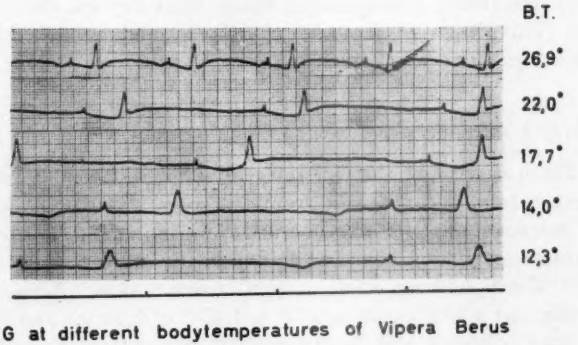

Fig. 5. Electrocardiograms recorded at different body temperatures in the viper, Vipera berus.

Time marks: 1 sec.

P-R interval.

As demonstrated from Fig. 6, the P-R intervals were always prolonged at lower body temperatures and lower heart rates. Most commonly the P-R interval was somewhat below the T-P line. Regarding the duration time of the P-R interval the extreme values measured in Vipera berus were 0.68 sec at b. t. 12.3° C, and 0.17 sec at b. t. 26.9° C. The figures in Tripodonotus natrix were 1.25 sec at b. t. 7.2° C, and 0.15 sec at b. t. 32.4° C. According to BIÖRCK and JOHANSSON (1955) the P-R values in frogs were 1.38 sec at b. t. 5.0° C, and 0.28 sec at b. t. 30.0° C, and in fishes 0.40 sec at 5.0° C, and 0.17 sec at 25.0° C.

The QRS complex.

In the lead used, the R spike was always positive, and the QRS duration became successively longer as the body temperature decreased. The amplitude showed a slight decrease at the lower body temperatures. An exponential relation between QRS duration and body temperatures was found (Fig. 7). The extreme values for duration times of the QRS complex in Vipera berus were: 0.12 sec at b. t. 12.3° C and 0.04 sec at 26.9°. In Tripodonotus natrix, the figures were: 0.18 sec at b. t. 7.2°, and 0.04 sec at 32.4°. The amplitudes ranged between 1.6 mV and 0.7 mV. In frogs and fishes, corresponding values after BIÖRCK and JOHANSSON (1955) were at b. t. 5.0°; 0.15 sec, and at 25°; 0.04 sec. The amplitudes were generally about 0.1 mV.

The Q-T interval.

Fig. 6 shows how the Q-T interval varies with changes in body temperature. It can be seen from this curve that the Q-T interval is subjected to the largest variations in length (BIÖRCK and JOHANSSON 1955.) Thus the Q-T duration in the viper at b. t. 12.3° C was 1.54 sec, while at 26.9° it was 0.52 sec. Tripodonotus natrix had a Q-T duration at b. t. 7.2° C of 2.15 sec and 0.28 sec at 32.4°. BIÖRCK and JOHANSSON (1955) reported the following values for Q-T duration in some other classes of vertebrates. Amphibians (frogs): b. t. 5.0° C; 2.36 sec, and 0.67 sec at 25°. Corresponding values in fishes were 1.16 sec at 5.0° C, and 0.18 sec at 25°.

Fig. 6

Fig. 7

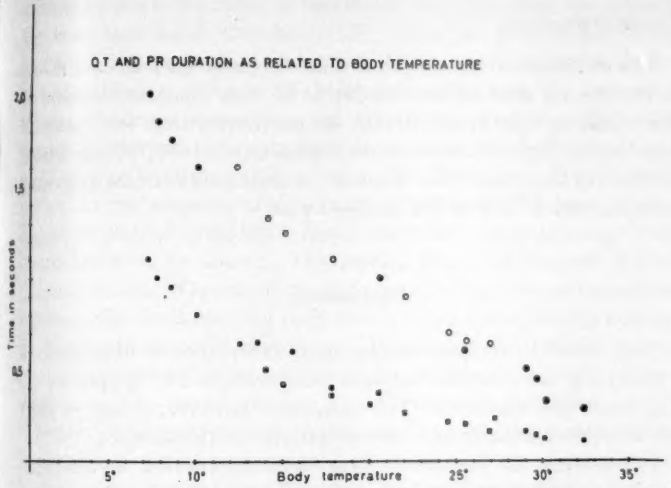

Fig. 6. Duration of Q-T interval (circles) and P-R interval (squares) plotted against body temperature of the animals.

Filled squares and circles: Tripodonotus natrix.

Open squares and circles: Vipera berus.

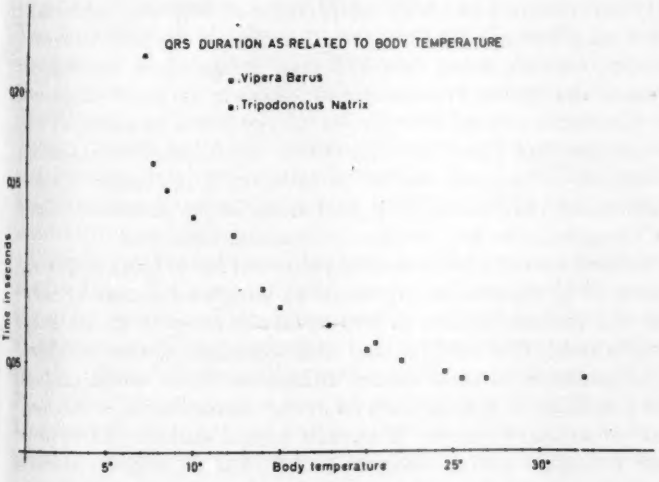

Fig. 7. Diagram showing the relation between QRS duration and body temperature.

Diastolic duration.

The duration of the electrical diastole under normal conditions in the species studied, turned out to be very short in relation to the whole cardiac cycle. It may be mentioned that the diastolic duration in Tripodonotus natrix makes out only $^1/_5$ of the whole cardiac cycle, while the diastole in human electrocardiograms occupies nearly half of the cardiac cycle.

Discussion.

The theoretical speculations and experimental observations concerning the relation between temperature and frequency of the heart are numerous. The literature, however, contains very contradictory reports and interpretations on this subject.

Concerning the vertebrate heart and its relation to temperature, KNOWLTON and STARLING's work from 1912 on the excised hearts of rabbits, is perhaps the first of importance. They stated that the rate of the isolated heart, perfused with normal oxygenated blood, was determined entirely by temperature, the curve showing the relation being a straight line. BARCROFT and IZQUIRDO (1931), presented several papers dealing with the connection between pulse rate and body temperature in frogs and mammals. They also brought forward the idea that there is a different relation between heart rate and body temperature in frogs in summer and winter. The seasonal variations in the heart's response to temperature seem, however, to be restricted upwards to the amphibian class. Thus BENEDICT (1932), found no hold for seasonal variations in his great work on metabolism in the larger reptiles. Neither did the present work, performed in the months of April to November, give any evidence for seasonal variations.

Generally it can be said that the regression line of heart frequency versus body temperature represents an integrated picture of nervous and humoral factors as well as factors intrinsic to the heart muscle cells. This complex and undeterminate picture is, therefore, suspected to show species differences which would exclude the possibility of putting forth an overall regression valid throughout the animal kingdom. Meanwhile several authors, KNOWLTON and STARLING (1912), SNYDER (1906), CLARK (1920), ADOLPH (1951), among others, claim that the regression in the intact

animal seems to be more or less linear over the range considered. On the other hand, CROZIER (1926), CROZIER, PINCUS and RENSHAW (1935), champion the view that the relation is not linear, but that the logarithm of the heart rate varies with the reciprocal of the absolute temperature, in accordance with the Arrhenius equation. Several attempts have been made to verify either one of these two hypotheses (BARCROFT *et al.* 1931). Lack of standardization in the experimental conditions together with lack of adequate statistical treatment have, however, prevented any firm conclusions to be drawn. The results from the present study, (Figures 2, 3 and 7) point distinctly toward a logarithmic regression between the heart rate and body temperature for reptiles belonging to the reptilian order Squamata. This was valid for all species studied and refers to the lengths of the electrocardiographic intervals, as well as to the numerical values of heart frequency.

Several different kinds of Q_{10} values or temperature coefficients have been found for biological processes, each corresponding to a distinctive mechanism of action. In the present connection it seems appropriate to distinguish between Q_{10} values around and above 2.0 and those considerably below 2.0. The former values are usually looked upon as an expression for thermochemical reactions, whereas low Q_{10} values express strictly physical processes, such as conductivity and rate of diffusion (GIESE 1957). It seems of interest to apply this scheme on the present study. The frequency of the heart involves chemical reactions, while components of the electrocardiogram, *e. g.* the duration of the Q-T interval, must be considered to be an expression of the rate of diffusion and conductivity in the ventricular myocardium. Thus it turned out that the Q_{10} values for the duration of the Q-T interval were considerably lower than the Q_{10} values for the heart frequency, respectively 1.54 and 2.10 in the temperature interval from 8—18° C.

Considering the steepness of the heart beat/temperature curve, the results from the present study are strongly opposing those reported by ADOLPH (1951), experimenting on turtles. While he arrived at ratios of heart frequency to body temperature of approximately 1 beat/°C/minute, the present study revealed ratios from 5—6 beats/°C/minute in a temperature interval from 8—32° C. This marked difference can be due to species differences or it may possibly be the consequence of adaptation or acclimatization.

Electrocardiographic results.

Electrocardiographic studies on poikilotherm animals cannot be intelligently interpreted without knowledge of the body temperature of the animals. As already mentioned, most of the earlier papers dealing with electrocardiograms of intact reptiles unfortunately lack such fundamental information.

Looking at Figures 4 and 5 certain characters are easily percepted. The long Q-T interval is very conspicuous when compared to the whole cardiac cycle. Thus the time taken for the wave to pass from the beginning of the P-wave to the summit of the T-wave in relation to the whole duration time for the cardiac cycle, is $^6/_8$—$^7/_8$. This extraordinary long Q-T interval is in strong contrast to the situation found in mammals, where the systolic duration amounts to around half of the entire cardiac cycle. The extraordinary long systolic duration was already noted by BUCHANAN in 1909. Unfortunately the publication contains no electrocardiographic tracings. In 1951, DAVIS *et al.* working with the Nile crocodile, also supported this finding. They stated that the electrical systole represents $^3/_4$ of the entire cycle in this animal.

Concerning the absolute values for duration times in the electrocardiograms, these are much like those reported by WILBER (1954) for the alligator. BIÖRCK and JOHANSSON (1955) studying the influence of temperature upon the hearts of amphibians and fishes, likewise presented results with great similarities to those from the present work concerning the duration times of the electrocardiographic intervals.

As for the heart rate/temperature relations, the various electrocardiographic components showed increased duration times upon lowering of the body temperature and a progressively shortening of the durations when the body temperature was elevated inside the temperature range used (5—35° C). This gross effect of temperature is reported by several authors (ASHMAN *et al.* 1945, NARDONE *et al.* 1955). The temperature effects upon the electrocardiograms of the species studied are demonstrated in Figures 4, 5, 6 and 7. The increase in length as the temperature was lowered of all intervals and the widening of the complexes are easily perceptible. Similar results were obtained for the frog heart by HOLTKAMP (1942). The underlying reasons for this phenomenon seems to depend on several factors working together in a complex

manner. The effect of temperature on the chemical reactions underlying the humoral impulse transmission in the heart may be one of these. Likewise changes in the cellular metabolism of the myocardium could modify the electrical properties of the heart.

I am specially indepted to cand. real. JOHN KROG for his kind supervision during all stages of this work.

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Effects of Alterations of Total Muscular Blood Flow on Local Tissue Clearance of Radio-Iodide in the Cat.

By

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Abstract.

HYMAN, C., S. ROSELL, A. ROSÉN, R. R. SONNENSCHEIN and B. UVNÄS. Effects of alterations of total muscular blood flow on local tissue clearance of radio-iodide in the cat. *Acta physiol. scand.* 1959. *46*. 358—374. — Simultaneous measurement was made of isotope clearance and of venous outflow or arterial inflow in the gastrocnemius muscle of anesthetized cats. The purpose was to explore if vasomotor nerves to the skeletal muscles might innervate different types of blood vessels. Restriction of the arterial inflow resulted in approximately proportionate decreases in flow and clearance. Stimulation of the sympathetic supply to the area caused a somewhat greater decrease in total flow than in clearance. Intra-arterial infusion of compound 48/80 or of methacholine, stimulation of the sciatic nerve or of the appropriate anterior roots, all increased flow and clearance pro-

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portionately. In contrast, stimulation of vasodilator pathway in the hypothalamus caused significant increase in flow with no consistent change in tissue clearance. With sympathetic stimulation, clearance of intra-arterially injected isotope showed a drop more nearly proportional to the drop in blood flow. None of the vasodilating procedures, however, increased the rate of clearance of intra-arterially administered isotope. It is concluded that most procedures influence the shunt and nutritional blood flow in a proportionate manner, but the active vasodilation resulting from hypothalamic stimulation is restricted to the shunt flow.

In recent years distinct vasoconstrictor and vasodilator innervation of the blood vessels of skeletal muscle has been described, chiefly on the basis of changes produced in the total blood flow of the muscle during appropriate electrical stimulation or reflex activation. It has not yet been possible to establish any specific physiological function for the vasodilator system, although several hypotheses have been discussed (Folkow and Uvnäs 1948, Uvnäs 1954). Furthermore, it is not known whether the dilator and constrictor fibres innervate different types of blood vessels, and what functional interrelationships between the two systems might exist on the peripheral level. The possibility that one or both sets of vasomotor nerves might influence a "by-pass" or shunt circulation was explored in the present study. The simultaneous measurement of total blood flow and of rate of electrolyte clearance (Kety 1949) from the muscle seemed to offer an approach to this problem: the latter determination might be taken as an index of the "nutritional" or capillary flow, while the former would include both this and "shunt" flow. Similar analysis of vasomotor changes induced by methods other than stimulation of the specific vasomotor nerves was necessary both to control the methods and to obtain corollary data. Important limitations on the usefulness of the clearance method were found; nevertheless, the inference could be made that the vasoconstrictor and vasodilator fibres innervate different sets of blood vessels, and that the vasodilator effect is primarily on the "by-pass" vessels.

Methods.

In most experiments male or female cats, weighing 2.0 to 4.0 kg were anesthetized with intravenous chloralose, 50 mg per kg body weight, supplemented with 20 % urethane as required, usually 3 to 7 ml. In

animals used for hypothalamic stimulation of the vasodilator pathway, urethane alone was used (total dose of 800 to 1,200 mg per kg) as this anesthesia generally favored a sustained response during stimulation. In a few experiments where urethane alone was used for procedures other than hypothalamic stimulation, it was established that the type of anesthesia did not alter the results. In all cases the trachea was cannulated and arterial pressure recorded from the carotid artery with a mercury manometer.

In the early experiments, venous blood flow from the gastrocnemius and associated muscles was recorded by cannulating the popliteal vein of one skinned leg and directing the blood through a photo-electric drop counter operating an ordinate recorder (LINDGREN and UVNÄS 1954). The blood re-entered the animal via the cannulated proximal stump of the same vein (in the figures: "venous flow"). In later experiments the blood entering the popliteal artery (in the figures: "arterial flow") was determined by recording with the silicone-filled drop counter of Lindgren (LINDGREN 1958). To insure against the participation of arterial collaterals to the gastrocnemius, blood was directed to the popliteal artery via the drop counter from the contralateral femoral artery. The internal iliac arteries and ipsilateral external iliac artery were ligated. To prevent clotting, heparin (25 mg/kg) was given intravenously in all experiments.

Occlusion of the arterial flow was accomplished by partially tightening a screw clamp directly on the femoral artery (when venous flow was measured) or on the plastic tubing of the cannula leading to the drop counter (when arterial flow was recorded). Electrical stimulation was produced by a square-wave generator of 1,000 ohms output resistance. The sympathetic chain, isolated and cut through the anterior approach, was stimulated at the level L4—L5 with a bipolar silver electrode at 1 to 10 cps and with an intensity adequate to produce the desired response, usually 1.0 to 4.0 V. Similar stimulation was applied to the sciatic nerve in its upper portion, or to anterior root L7 or S1 isolated intradurally. For stimulation of the hypothalamic portion of the sympathetic vasodilator pathway (LINDGREN *et al.* 1956), a unipolar, stainless steel electrode was oriented stereotaxically to the point of optimal response. An acceptable response consisted of an increase in blood flow without an associated rise in arterial pressure, and subsequent abolition of the hyperemia with atropine, usually 0.2 mg/kg. The parameters for hypothalamic stimulation were generally 30 to 50 cps and 1.5 to 3.0 V. As a rule, the stimulations lasted 4 min, to allow adequate time for the establishment of a measurable clearance rate under the experimental conditions.

Methacholine (acetyl-β-methylcholine-iodide. "Mecholyl") 0.08—0.2 μg/min and compound 48/80 (condensation product of p-methoxyphenethyl-methylamine with formaldehyde) 30—50 μg/min (ROSÉN, STRANDBERG and UVNÄS 1957) were infused either into a plastic cannula introduced into a sidebranch of the femoral artery or into a side arm of the arterial drop counter, at a rate adequate to produce the desired response. Atropine and dihydroergotamine were given by intravenous

infusion. The situations in which each of the above agents was used are indicated with descriptions of the experimental results. Dextran (Macrodex, Pharmacia) was given intravenously as required to compensate for blood loss. In experiments requiring spinal laminectomy, 10 to 15 ml of 5 % dextrose was administered intravenously or subcutaneously following the operative procedure.

Rectal temperature, continuously monitored with a mercury thermometer, was maintained at 36—37° C by means of an infrared lamp directed toward the animal's chest and abdomen. In the later experiments, temperature of the gastrocnemius muscle was measured with a thermocouple needle inserted directly into the muscle. Except in those experiments where it was desired to alter the muscle temperature, the latter was maintained at 35—37° C with an external heat lamp.

The source of radioactivity in all but a few experiments was a solution of $Na^{131}I$ (specific activity of ^{131}I: 50—150 μc per 0.1 ml in 0.9 % NaCl). In nine experiments $^{24}NaCl$ was used; the results with this isotope were the same as with $Na^{131}I$. Exactly 0.1 (in a few cases 0.2) ml of the solution was injected by syringe into the body of the gastrocnemius muscle using a 26 gauge hypodermic needle ("intramuscular isotope injection"). Alternatively, in those experiments designated as "intra-arterial isotope injection", the material was injected into the popliteal artery via a side arm of the arterial drop counter (cf. Dobson and Warner 1957). To reduce the amount of isotope which would enter the general circulation in these experiments, a heavy thread was first placed loosely around the lower part of the inferior vena cava and all branches between this thread and the common iliac veins were tied. A large polyethylene cannula was placed in the femoral vein contralateral to the side on which blood flow and clearance were to be determined. This cannula was directed centrally with its end in the common iliac vein almost to the vena cava; the cannula was tied in place and its distal end clamped. Just prior to the intra-arterial injection of isotope, the thread around the vena cava was pulled taut to occlude the cava. The isotope was then injected in 5 to 15 sec. Following a further period of 15 to 20 sec, the clamp on the cannula in the contralateral common iliac vein was released, and the venous blood was collected for about 30 sec. The clamp was then replaced on the cannula and the caval occlusion was released. The collected blood was discarded and an equal amount of dextran was injected intravenously to compensate for this loss. In this way, all venous blood from the experimental leg during the isotope injection and for 30 sec thereafter was removed from the animal, and along with it the greater part of the isotope which had passed through the leg.

The radioactivity in the leg was monitored by a Geiger-Müller tube (Tracerlab, TGC 8) or a scintillation detector (Philips, PW 4111), connected to an appropriate counting rate meter (NRD-CH-1200), and the output was recorded continuously on a direct writing oscillograph. The detector was mounted directly over the injection site in such a way as to "see" a constant volume of tissue (Widmer and Staub 1958). When using the scintillation detector a lead collimator restricted

the amount of tissue "seen". The counted area had then a diameter of about 10 cm at intra-arterial isotope injection and about 1.3 cm at intramuscular injection. When using the G. M. tube this was shielded with lead so that the counted area had a diameter of about 4 cm. Results with the two detectors were identical. Clearance rates were determined from semilogarithmic plots of the raw data after correction for background activity. The slope of the clearance curve was expressed as %/min (KETY 1949).

After some intramuscular isotope injections, the calculated rate of clearance was so low and the final "background" level was so high as to suggest that the material had been injected into an inactive area where the isotope remained as a depot unaffected by physiological processes. When this occurred the experiments were discarded. After intra-arterial isotope injections, certain vagaries in the clearance curve, described later, often occurred. The results of these experiments were not used. Likewise, in any case where the clearance rate *following* an experimental procedure did not return to the control rate along with the blood flow, the data were not included among the general results. When the animal's general condition remained good, a second, and occasionally a third, isotope injection was made and the experimental procedure repeated.

Results.

Since the results of the intramuscular and intra-arterial injections of isotope were different, they are presented separately.

I. Intramuscular isotope injection.

a. Arterial clamping.

Reduction in arterial blood flow to between 80 % and 0 % of the control value was uniformly accompanied by a decrease in clearance rate of approximately the same percentage (Fig. 1). It is interesting to note that the results from the intravenous and the arterial blood flow recordings appear to group themselves separately. The variations within each of the two groups are about the same. Complete occlusion was performed in many experiments as a test for the existence of collateral vessels; this always reduced the clearance to zero.

b. Reactive hyperemia.

Upon restoration of flow after a complete arterial occlusion of 4 to 5 min duration, reactive hyperemia lasting $^1/_2$ to 2 min occurred. Generally, a tendency for increase of the clearance rate

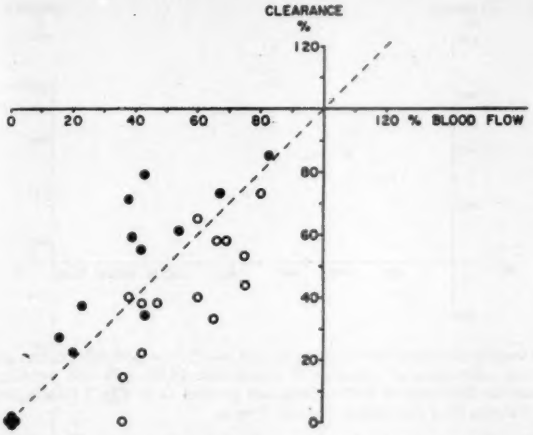

Fig. 1. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during clamping of the arterial inflow. Intramuscular injection of $Na^{131}I$. Data are plotted as percentage of the initial resting values. Each point represents a separate trial. The points at (0.0.) represent a larger number of trials.
○ — Venous flow recording; 14 trials in 9 cats. 2 isotope injections were made in 1 cat.
● — Arterial flow recording; 16 trials, 11 cats. 2 isotope injections were made in 1 cat.

above the control could be seen during this time. The response was not of sufficient duration to allow accurate determination of the clearance rate in the same manner as has been done for the results of other procedures.

c. Compound 48/80 and methacholine.

The intra-arterial infusion of the histamine releasing substance compound 48/80 (dose: 30—50 μg/min) produced an increase in blood flow and clearance rate as shown in Fig. 2. A similar response was produced by methacholine (0.08—0.2 μg/min, Fig. 3). These two sets of data are not comparable quantitatively since the former were obtained with the intravenous recording technic and the latter with the intra-arterial.

d. Sciatic and anterior root stimulation.

TLe effects of increased muscular activity produced by stimulation of the sciatic nerve are shown in Fig. 4. In general, an increase in both clearance rate and blood flow occurred. The quantitative

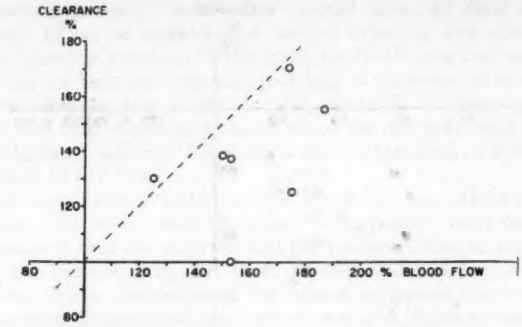

Fig. 2. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during intra-arterial infusion of compound 48/80. (30—50 μg/min.) Intramuscular injection of $Na^{131}I$. Data are plotted as in Fig. 1 (see legend).
○ — Venous flow recording; 7 trials, 4 cats.

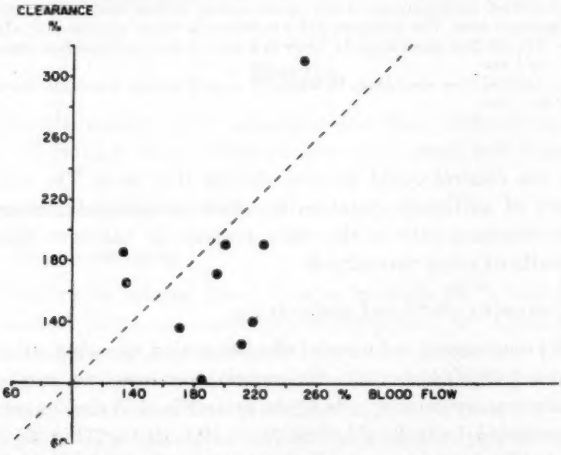

Fig. 3. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during intra-arterial infusion of methacholine (0.08—0.20 μg/min). Intramuscular injection of $Na^{131}I$. Data are plotted as in Fig. 1.
● — Arterial flow recording; 10 trials, 5 cats. 2 isotope injections were made in 2 cats.

relations between these two responses varied more widely during venous flow registration than during arterial, but the trends of the two were similar. The prior administration of atropine (0.5

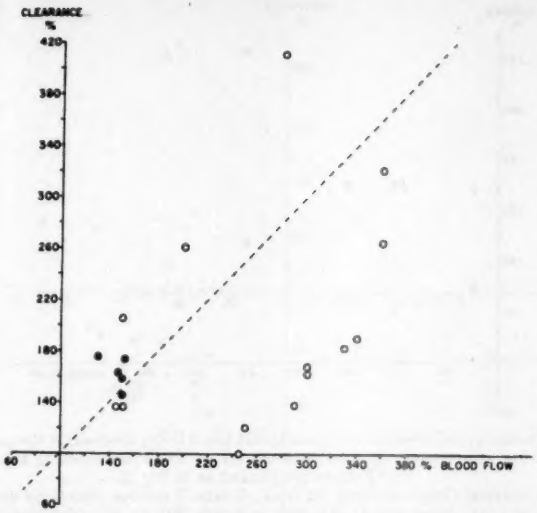

Fig. 4. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during stimulation of the sciatic nerve. Intramuscular injection of $Na^{131}I$. Data are plotted as in Fig. 1.
○ — Venous flow recording; 14 trials, 6 cats. 2 isotope injections were made in 4 cats.
● — Arterial flow recording; 5 trials, 4 cats.

mg/kg) with or without dihydroergotamine (0.5 mg/kg), given to block the action of vasomotor fibres in the sciatic nerve, was without apparent effect.

A separate series of experiments was performed in which the anterior root (L7 or S1) was stimulated instead of the sciatic nerve in order to avoid the stimulation of any sympathetic fibres; the preganglionic fibres arise no lower than the level of L4 or L5. These experiments (Fig. 5) gave essentially the same results as those with sciatic stimulation.

e. Sympathetic chain stimulation.

Appropriate unilateral stimulation of the sympathetic chain between L4 and L5 produced a vasoconstriction and reduction in blood flow that was accompanied by an extremely variable change in clearance (Fig. 6). On occasion the clearance rate even increased in the face of a decrease in blood flow. Atropine (0.5

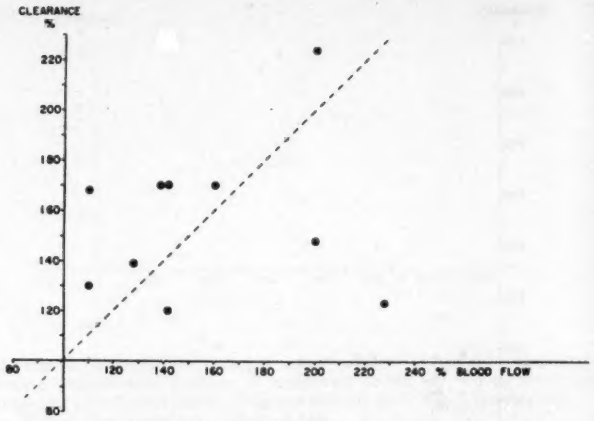

Fig. 5. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during stimulation of anterior roots L7 or S1. Intramuscular injection of $Na^{131}I$. Data are plotted as in Fig. 1.

● — Arterial flow recording; 10 trials, 5 cats. 3 isotope injections were made in 1 cat. (Note: Points (200, 150) and (200, 220) are from the same clearance curve.)

mg/kg), given in order to block cholinergic vasodilator activity, did not decrease the variability of results. In addition, no great difference in the spread of the values was apparent in comparing the results of venous flow recording with those of arterial. It is of some interest, however, that almost all of the points lie above the line which indicates a clearance/blood flow ratio of one. That is, that for a given intensity of stimulation, reduction in blood flow generally was proportionately greater than reduction in clearance rate.

The possible effect of muscle temperature on the clearance/blood flow ratio during sympathetic stimulation was investigated in five animals by stimulating while the muscle was either cooled with an ice bag to 27—28.5° C or warmed with a heat lamp to 35—37° C; in 3 of the 5 experiments trials were made in both temperature ranges. No consistent differences in the ratios were noted between the two temperature levels; in all cases the reduction in clearance during sympathetic stimulation was proportionately less than the reduction in blood flow, in consonance with the general trend of the larger series of trials in which temperature was not controlled.

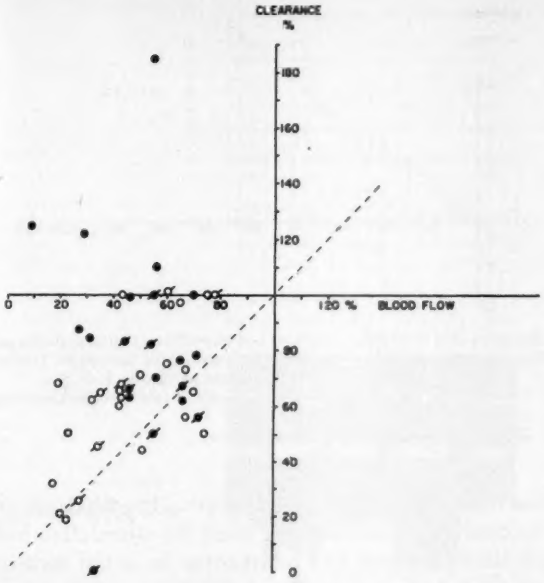

Fig. 6. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during stimulation of the ipsilateral sympathetic chain at the level of L4—L5. Intramuscular injection of $Na^{131}I$. Data are plotted as in Fig. 1.
○ — Venous flow recording; 0.5 mg/kg atropine i. v. given before stimulation.
○ — Venous flow recording; no atropine.
● — Arterial flow recording; 0.5 mg/kg atropine i. v. given before stimulation.
● — Arterial flow recording; no atropine.
Venous recordings represent 25 trials in 14 cats. Arterial recordings represent 21 trials in 16 cats; 2 isotope injections were made in 1 cat.

f. Hypothalamic vasodilator stimulation.

In contrast to the findings with the other procedures studied, sympathetic vasodilator stimulation in most cases caused no change in rate of clearance even with relatively large increases in blood flow (Fig. 7). Variation in results is seen as before. That the failure to obtain a change in clearance was not the result of error in technic is indicated by findings such as shown in Fig. 8 where, in a single preparation, an increase in clearance rate occurred during intra-arterial administration of methacholine (D in Fig. 8), while no such change occurred during hypothalamic stimulation (B in Fig. 8), even though there were comparable increases in blood flow in the two cases. In a few cases, where

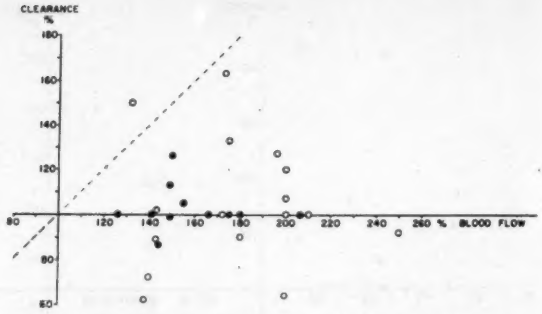

Fig. 7. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius during stimulation of the hypothalamic vasodilator pathways. Intramuscular isotope injection of $Na^{131}I$. Data are plotted as in Fig. 1.
○ — Venous flow recording; 16 trials, 12 cats. 2 isotope injections were made in 1 cat.
● — Arterial flow recording; 11 trials, 5 cats.

there was no change in clearance rate during hypothalamic stimulation, the clearance rate decreased when the stimulation had stopped and the blood flow had returned to its initial control level. Such an example is seen in Fig. 8.

II. Intra-arterial isotope injection.

In the hope of obtaining results more uniform than those after intramuscular injection, some of the experiments were repeated with the technic of intra-arterial injection of isotope. It was noted that in general the initial clearance rates were considerably higher than following intramuscular isotope injection. Furthermore, in many of the trials the semilogarithmic plot of the initial clearance rate showed an inflection instead of being straight as was the usual case after intramuscular injection. These experiments were not included among the present data. As can be seen in Fig. 9, none of the procedures that augmented blood flow increased the clearance rate; instead there was sometimes a decrease. Stimulation of the sympathetic chain, however, gave a change in clearance in the same direction as blood flow changes. These latter results differed in two major respects from those after intramuscular injection (Fig. 6). First, the variability seemed to be much less. Secondly, the trend of the values was much closer to a clearance/blood flow ratio of one.

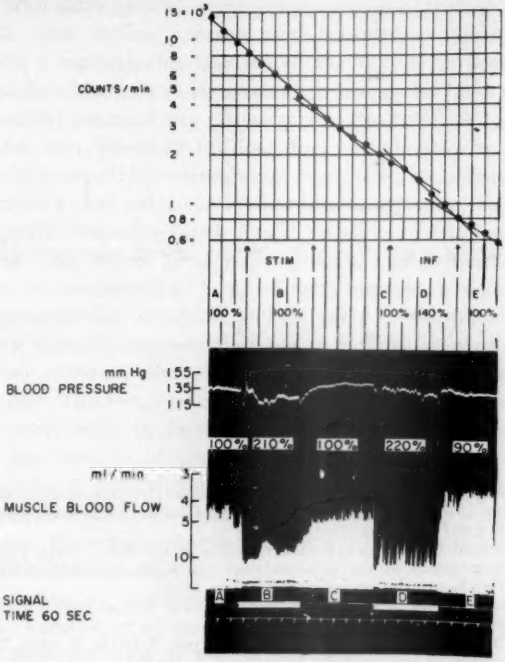

Fig. 8. Cat 2.5 kg. Urethane.
The effect on clearance rate and blood flow in the gastrocnemius and associated muscles of the right hind leg of stimulation of the sympathetic vasodilator pathways in hypothalamus and of infusion of methacholine.
B. Stimulation, 3.0 V, 50 imp/sec, 5 min.
D. I. a. infusion of 0.5 μg methacholine, 5 min.
Note that there is no change in clearance rate during vasodilator stimulation, as contrasted with the increase during methacholine infusion.

Discussion.

The results of these experiments raise several questions regarding the validity of clearance rate as a measure of labelled electrolyte turnover in muscle. If the results of the intramuscular injection be considered first, it appears that the clearance rates and blood flow generally change in the same direction during all procedures except that of vasodilator stimulation. Common to all groups of experiments, however, is a high degree of variability for which several possible reasons may be adduced. Foremost, perhaps, is the random non-uniformity of the tissue into which the electrolyte

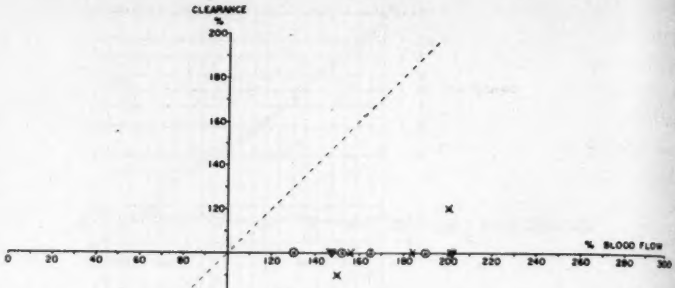

Fig. 9. Change in clearance rate (ordinate) and blood flow (abscissa) in the gastrocnemius. Intra-arterial injection of $Na^{131}I$. Data are plotted as in Fig. 1. Arterial flow recording.
● — Stimulation of the sympathetic chain at L4—L5; 7 trials, 7 cats.
⊙ — Stimulation of the hypothalamic vasodilator pathway; 5 trials, 4 cats. Two isotope injections were made in 1 cat.
× — Stimulation of the sciatic nerve; 7 trials, 6 cats. Two isotope injections were made in 1 cat.
▼ — Intra-arterial infusion of methacholine; 3 trials, 2 cats. Two isotope injections were made in 1 cat.

solution was injected in each case: the ratio of active muscle to connective tissue might differ with each injection. If the vasculature of these two tissues should differ in respect to permeability, innervation or response to drugs, a given change in total blood flow would give differing changes in clearance rates during the several procedures, depending on the distribution of the injected electrolyte between the tissues. Another difficulty inherent in the intramuscular injection technic is the pressure produced by the volume of fluid which might affect local vasomotor nerves or the blood vessels themselves. Since it is from precisely this area that the clearance is measured, such effects would be critical (cf. KETY 1953). Indeed, WARNER and DOBSON (1953) demonstrated a profound influence of injection volume on apparent clearance rate.

Because of these possibilities, attempts were made to measure

clearance after intra-arterial administration of the labelled electrolyte. With this technic, muscle and connective tissue would each receive a reproducible fraction of the isotope in proportion to its respective blood flow, its associated interstitial fluid volume and its vascular permeability (DOBSON and WARNER 1957). These expectations were partially fulfilled, for, as indicated in Fig. 9, the results of sympathetic stimulation were more consistent under these conditions than after the intramuscular injection; the trend of these results was also much closer to a clearance/blood flow ratio of one. The failure to increase clearance as measured with this technic, as contrasted with the uniform increase demonstrated by the intramuscular injection technic after the application of vasodilating stimuli, suggests that under conditions used in the intra-arterial method, the clearances represent some kind of maximum rate. This result would be expected if the injected isotope distributes itself locally in the tissue fluid irrigated by the capillaries patent at the moment of injection. Opening more capillaries in other parts of the muscle would not be expected to increase the rate of removal of the labelled material from the already maximally perfused area. Obviously, decreases associated with vasoconstriction would still be detectable.

Both technics of isotope administration thus have severe limitations. Variability of response after intramuscular injection is so great as to preclude its use for quantitative purposes in the individual case, especially in animal experiments. On the other hand, the intra-arterial technic, at least as used here, can only be used to detect decreases in clearance rates. When a sufficiently large number of trials is made it is possible to detect a trend, and some tentative conclusions may be made as to the physiological significance of the results. Our findings in most of these experiments are consistent with the postulate that the rate of clearance is an index of the capillary or nutritional blood flow (KETY 1949). Where the change in clearance is proportional to the change in total blood flow, the procedure probably causes proportionately equal change in flow in both nutritional and non-nutritional vessels. This appears to be the case with all of the stimuli examined except for the activation of the sympathetic chain and the vasodilator pathway. Similar correspondence between clearance and directly measured total blood flow through muscle has recently been reported by three groups (PRENTICE *et al.* 1955, WALDER 1955, and SOKOLOFF cited by KETY 1953).

All of these studies used modification of perfusion pressure to change the total flow through the tissue.

Two characteristics of the results of sympathetic stimulation, with the intramuscular technic, should be considered. First, the fact that the variation is greater than with the other experimental procedures might be explained if different groups of vasomotor fibres with different functions were stimulated in the various trials. For example, there might be independent constrictor fibres to nutritional and shunt vessels. Any significant role of the cholinergic vasodilator fibres in this variable response to sympathetic stimulation was ruled out since the administration of atropine had no apparent effect on the results. Secondly, despite the variability, the majority of the sympathetic stimulation experiments showed changes in clearance less than the associated decrease in blood flow. This would suggest that a large number of constrictor fibres in the sympathetic chain affect non-nutritional vessels whose closure would tend to reduce flow but not clearance. The above interpretations are not supported, however, by the results obtained after intra-arterial isotope administration. These results are much more uniform and the points indicate a clearance/blood flow ratio of one. The factors involved in the clearance determination with either technic are too poorly defined to allow an analysis and interpretation of these divergent findings.

The failure of hypothalamic vasodilator activation to alter clearance rate in the intramuscular isotope series is especially significant, since all other vasodilator procedures, such as reactive hyperemia, administration of compound 48/80 or methacholine, and sciatic or anterior root stimulation *did* increase clearance. To the extent that the clearance determinations are valid, this result indicates that the vasodilator fibres influence non-nutritional vessels, principally. That is, one may postulate vascular shunts in muscle which are opened by activity of the vasodilator fibres, increasing total blood flow without an increase in electrolyte clearance or tissue nutrition.

Similar shunts under independent nervous control are well known in skin. The studies of BÁRÁNY (1955) using technics analogous to those used in the present works, showed, in fact, a competition between the nutritional and shunt flow for the limited blood supply to this tissue. His studies give further support to the use of tissue clearance technic in assessing the nutritional circulation.

In recent years, the possibility of a dual blood flow in skeletal muscle has been pointed out by several authors using different technics. BEAVERS, COVINO and RENNIE (1956) studied the factors responsible for the increased femoral blood flow in the hypothermic dog. They found that the peripheral vasodilation was mediated by cholinergic sympathetic fibers. In another study, the same authors (COVINO, BEAVERS and RENNIE 1956) suggested that opening of A-V shunts in skeletal muscle was responsible for the increased blood flow. HONIG and GABEL (1958) used microelectrodes for polarographic measurement of tissue oxygen "tension" in muscle and skin, along with a Clark electrode in the femoral vein to study the redistribution of flow between nutrient and non-nutrient channels. The experiment was done on isolated dog hind-limbs perfused at constant flow. They concluded among other things, that epinephrine and norepinephrine redistributed blood from non-nutrient into nutrient channels in muscle. RAPAPORT *et al.* (1952) in a study of the circulatory changes in the human calf and toe after lumbar sympathetic blockade, presented evidence for the *lack* of influence of this procedure on nutritive flow through muscle. In view of the data of BARCROFT (1943) on the increase in total blood flow through the muscles of the human calf after such a block, it was suggested that there are indeed shunt vessels in muscle, and that these appear to be controlled by the vaso-constrictor outflow.

From the morphological point of view, ZWEIFACH (1957) and SAUNDERS (1957) have shown arteriovenous shunts in skeletal muscle. In contrast, DIETER (1954) showed that spheres greater than 19 micra in diameter could not pass through normal muscle blood vessels and so concluded that there are no shunts in muscle. HYMAN (1957) pointed out that shunts of any particular morphological characteristic are not a necessary prerequisite for the hypothesis of a "non-nutritive" by-pass circulation.

What physiological role might be played by such a special vascular mechanism is wholly speculative and must await future investigations for elucidation.

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